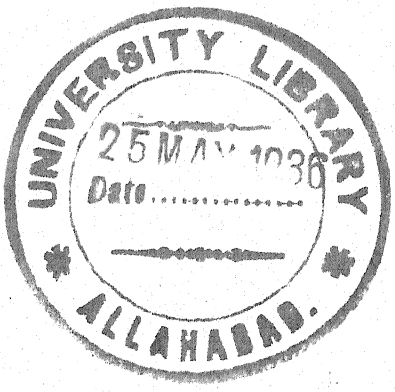
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ERRATA

Page 38, WEISS abstract, line 14, *read* discomycetous *for* iscomycetous.

Page 240, heading of paragraph 3, *read* spraying *for* spring.

Page 253, line 12 *read* Tuberculina *for* Tubercularia.

Page 343, line 8, *read* from *for* for.

Page 369, line 3 from bottom of page, *read* copper *for* oil.

Page 410, line 20, last word, *read* noninoculated *for* inoculated

Page 439, line 2 *read* diseased *for* dismissed.

Page 443, line 22, *read* participle *for* principle.

Page 480, line 7, *read* virus preparation multiplied by 100, is also given *for* virus preparation, is also given.

Pages 491 and 492, heading of column 11, table 2, *read* 26.78 *for* 26.27.

Page 514, line 8, *add* All 4 forms of *T. levis* tested, infected rye.

Pages 603, line 21, *read* bandage gauze *for* cheesecloth.

Page 604, table 1, column 1, *read* $.4 \times 10^{-1}$ *for* 4×10^{-1}.

Page 856, line 3, *read* on to the surface *for* on the surface.

Page 857, line 36 *read* value *for* values.

Page 955, line 13, *read* 15 to 40 *for* 15 or 40.

Page 1115, line 15 *read* or X virus *for* of X virus.

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Hybridization Between Ustilago hordei and U. medians. C. C. ALLISON.

Six barley varieties were inoculated with intraspecific and interspecific combinations of monosporidial lines of *Ustilago hordei* and *U. medians*. The pathogenicity of the interspecific combinations was similar to that of one or the other of the intraspecific combinations, except on Peatland barley, on which they produced no smut, whereas the combinations of lines of *U. medians* smutted 9 per cent of the heads. Four combinations were made of F_1 monosporidial lines from a *U. hordei* × *U. medians* cross. All of the resulting F_2 dicaryophytes were distinct in their pathogenicity to the 6 varieties of barley. Two were more pathogenic to Velvet barley than the F_1 dicaryophytes or either of the intraspecific combinations. In the F_1 the head type was intermediate and the chlamydospores were echinulate. In the F_2 there was segregation for compact, loose, and intermediate head type and for smooth and echinulate chlamydospores. Results indicate also that factors for sex, pathogenicity, head type, and chlamydospore-wall characters can be segregated independently.

Black Stele Root Rot of Strawberry. H. W. ANDERSON.

A wilt of the strawberry has caused serious losses in large commercial plantings in Eastern Illinois. During the first bearing season the diseased plants begin to wilt shortly before the first berries ripen. No evidence of the disease has been found during the first growing season. The disease usually appears first in areas where the water-holding capacity of the soil is high, but later may spread to well drained loam soil. The stelar tissues of the roots of the diseased plants are dark brown to black and contain numerous oospores. The cortical tissues may appear normal, but few secondary roots are produced. Wilting results from the fact that the conductive tissues are destroyed. The fungus associated with the disease has been isolated but has not produced conidia in culture or on diseased roots. It is thought to be a Phytophthora. Black stele has been found in widely separated localities in Illinois and neighboring States. It seems to be similar to the Lanarkshire disease of Scotland.

Phytophthora Trunk Canker of Apple. R. C. BAINS.

Phytophthora cactorum has been recognized as the cause of a destructive disease of the trunks of apple trees in Indiana. In 1933 the disease was serious on 12- to 18-year-old Grimes trees. In 6 commercial orchards from 43 to 76 per cent of the trees of this variety were partially or completely girdled. An irregular canker frequently involves the entire trunk and bases of the scaffold branches. The first outward symptom is a wet area appearing on the trunk. Both the bark and sapwood are invaded by the fungus, which in the bark is both inter- and intracellular. *Phytophthora cactorum* has been isolated from 48 diseased trees. The disease has been reproduced on the trunks of 15 Grimes trees of ages 8, 12, and 15 years, following inoculations with mycelium from a pure culture of the fungus. Inoculations made on the limbs resulted in no infection. An 8-year-old Rome tree also was infected by artificial inoculation. No infection was obtained from inoculations made on the trunks of Delicious, Winesap, Jonathan, Liveland, Hiburnal and Virginia Crab trees. Thirteen cankers were treated by scarification with the resulting successful removal of the infected areas from 10 trees.

Importance of Sanitation in Controlling Crown Gall of the Red Raspberry. W. M. BANFIELD and E. C. MANDENBERG.

Mature first-year plants without visible crown-gall infection were selected in the late autumn from fields wherein from 0.1 to 84 per cent of the plants were galled. They were healed in between the rows from several hours to days, then removed, disin-

fected, and stored over winter in virgin sand. Several lots of these apparently clean plants from the 0.1 per cent diseased field were treated as follows: exposed to inoculum or mixed with gall plants and stored in virgin sand, stored in fields habitually used by nurserymen for over-winter storage of bramble-fruit plants, in fields from which diseased plants had recently been removed, or in inoculated sand. All plants were grown throughout the following season in a clean field. Galls developed on 2.6 per cent of the plants taken from the best (0.1 per cent visibly galled plants) field, on 93.7 per cent of those from the worst (84 per cent). Respectively, 40 to 82 per cent of those stored in diseased fields developed gall, 41 to 79 per cent of those held in nursery storage grounds developed gall, 63 per cent of those mixed with galled plants and stored in virgin sand developed gall and 63 per cent of those stored in inoculated sand developed gall.

Tobacco Wildfire Control in Pennsylvania. W. S. BEACH.

Transplanting disease-free tobacco plants to isolated plots that had become contaminated by wildfire a year before shows that *Bacterium tabacum* lives at least a year under field conditions in Pennsylvania and that inoculum from field sources is important in the annual development of the disease. Field observations and seed-bed tests indicate that incompletely decayed butts, stems, etc., of the previous tobacco crop harbor the parasite. In searching for unknown hosts of *Bact. tabacum,* spots characteristic of wildfire, yielding cultures of *Bact. tabacum,* and centering around the flea-beetle injuries, have often been found upon the common weed, *Physalis virginiana,* growing next to wildfire-infected tobacco. This weed is not known to be a host in the absence of tobacco. In experiments on seed beds contaminated with old infected tobacco, it has proved feasible to apply Bordeaux mixture at seed-time with either nonsprouted seed or that sprouted in bulk. Only by this early treatment has it been possible to obtain field plots free of wildfire. In similar experiments, dusting with copper-lime dusts was less effective than Bordeaux mixture. A spray made of calomel and milk powder gave control comparable to Bordeaux mixture, but the calomel was commonly injurious to the tobacco root systems by hindering the development of lateral roots near the soil surface.

A Mosaic on Cabbage in Wisconsin. L. M. BLANK.

Cabbage, *Brassica oleracea* var. *capitata,* plants brought to seed in the greenhouse in the sping of 1934, from heads grown in the field in 1933, showed a high percentage of mosaic. Field observations in August, 1934, established the fact that, in the intensive cabbage section of southeastern Wisconsin, mosaic was generally prevalent on cabbage. Transmission has been successfully accomplished by use of aphids and by plant extract. The symptoms of mosaic on cabbage seed plants in the greenhouse vary from faint to prominent mottling, followed by necrosis and dropping of the lower affected leaves. The effect of mosaic on seed production has not been definitely determined, although some of the affected seed plants produce abundant seed, others little or no seed. Distinct symptoms of mosaic in commercial cabbage fields became increasingly evident during the latter part of the growing season. Plants affected early were usually stunted and retarded in maturity, whereas those affected later in the season were less severely injured. Mottling of leaves was accompanied by an apparent change in the bloom frequently followed by necrosis in local areas on outer leaves and head leaves.

Pathogenicity and Physiology of Pseudopeziza ribis. E. C. BLODGETT.

Anthracnose of *Ribes* spp. and its causal organism *Pseudopeziza ribis* have been investigated by the writer since 1931. Seven isolates of the fungus from Wisconsin, Oregon, Canada, and The Netherlands have been studied comparatively in regard to

morphology, physiology, and pathogenicity. Considerable variation was found in the size and shape of the crescent-shape conidia produced by the isolates in culture and on the host. Microconidia of isolate 2 were over twice the length of those of isolate 4. The optimum temperature for germination of conidia and for growth was about 20° C. Ascospores germinated best at about 12°. Optimum pH of the media used for spore germination and growth was approximately 5.3–7. Infection studies, using conidia (principally) and ascopores, on over 400 plants have shown that: isolates derived from currant are more pathogenic on currant than on gooseberry and *vice versa;* the fungus penetrates directly through either leaf surface; less vigorous hosts are relatively more susceptible; all common garden varieties may become severely infected. The life history of the fungus has been followed in some detail and correlated with epidemiology and control of the disease. The ascigerous stage on currant and gooseberry was found in abundance.

Evidence of the Seed-Borne Nature of Late Blight (Phytophthora infestans) of Tomatoes. O. C. BOYD.

Late blight has not been injurious to tomatoes in Massachusetts since 1905 until the outbreaks of 1932 and 1933. Summer-weather conditions alone seemed not to account for its behavior in 1932 or 1933. Also, there appeared to be no definite relation between its occurrence on potatoes and on tomatoes. An experiment at Amherst in 1934 indicated that *P. infestans* overwintered in tomato seed saved in 1933 and thereby initiated primary infection in the 1934 crop. This occurred both in transplants grown in the greenhouse and in plants from seeds sown in the field at transplanting time. Control plants from healthy seeds did not show primary infection. Assumption regarding the seed-borne origin of the disease was strengthened when it was discovered that diseased seeds from both the 1933 and 1934 crops contained mycelium outside and inside the seed coats and yielded pure cultures of *P. infestans* on agar. Outbreaks of late blight in 1932 and 1933 might perhaps be explained, at least in part, by the more favorable overwintering of the fungus in tomato seeds on farms during the 2 preceding, unusually mild winters. The disease was not important in 1934, in spite of a cool and unusually wet September.

Microorganisms Infecting Pines Attacked by Dendroctonus frontalis. W. C. BRAMBLE and E. C. HOLST.

The southern pine beetle, *Dendroctonus frontalis,* introduces certain microorganisms into the bark of trees that it attacks. These microorganisms invade the sapwood and appear to be a primary factor in the rapid dying of attacked trees. An unnamed basidiomycete, an ascomycete identified as *Ceratostomella pini,* and a new species of yeast, belonging to the Zygosaccharomyces, are carried by the beetle and are the first to invade the sapwood. The basidiomycete penetrates rapidly into the outer growth rings and is abundant during the first week of attacks. Penetration by *C. pini,* and the yeast follows closely on that by the basidiomycete. By the second week they are penetrating as deeply as the latter into the sapwood. During the final stages of the attacks, *C. pini* becomes the most prominent microorganism in the sapwood. Both the basidiomycete and *C. pini* are capable of killing trees, when used for inoculations, provided that the inoculation points encircle the stems of the trees.

Streak, A Virus Disease of Roses. PHILIP BRIERLEY.

A virus disease producing brownish or reddish patterns of ring or vein-banding types in leaves or ring patterns in canes is common in roses at Arlington Experiment Farm, Virginia. Its presence has been disclosed also in the District of Columbia, Beltsville, Maryland, and New York City, N. Y. The understocks Multiflora and Odorata

and varieties of the Tea, Hybrid Tea, Hybrid Perpetual, Hybrid Rugosa, Hybrid Wichuriana, Hybrid Multiflora, Polyantha, Hybrid Bengal, China, and Noisette classes have been found naturally infected. On experimental inoculation by budding, certain hybrid teas, such as Madame Butterfly, develop necrotic areas about the inserted bud. Commonly the stem is girdled at the site of the bud, the distal parts die and the leaves wither but persist. Frequently, nearly black secondary lesions with sharply defined margins, not depressed, appear on young lateral branches below the inserted bud. The virus has been transmitted by tissue union only, not by juice inoculations. Streak is clearly distinct from rose mosaic and apparently distinct from the rose wilt and dieback described by Grieve in Australia.

Symptoms of Rose Mosaic. PHILIP BRIERLEY.

Rose species, understocks, and varieties exhibit a wide range of chlorotic leaf symptoms suggestive of virus infection. Few of these chlorotic types will produce mosaic symptoms on budding to Madame Butterfly or other hybrid teas susceptible to White's rose mosaic. The chlorotic leaf symptoms of White's rose mosaic, proved for various types of roses, scarcely exceed in range those shown by Madame Butterfly alone, namely, local puckering and chlorosis associated with the veins, oakleaf, and etch types, and chlorotic ring-spotting. Among the virus-like types not transmitted to Madame Butterfly by budding are the conspicuous and prevalent crinkle types in Manetti. Symptoms produced in Manetti by budding to White's rose mosaic are less prominent than in Madame Butterfly, but comparable to Briarcliff symptoms. These symptoms in Manetti have thus far been recognized only under glass, and will probably be difficult to identify under field conditions. A yellow variety of mosaic differing from White's rose mosaic chiefly in producing chlorosis of a brighter and lighter yellow has been found in Ontario, New York, Maryland, and Virginia on Hybrid Tea, Hybrid Perpetual and Hybrid Rugosa types. Neither White's mosaic nor the yellow type has been transmitted by methods other than tissue union.

Heterothallism in Peronospora parasitica. HELENA L. G. DE BRUYN.

Pure cultures of the obligate parasite—*Peronospora parasitica*—each derived from a single spore were obtained by inoculating sterile Brassica seedlings on agar slants. Eleven single spores were isolated, originating partially from different places and varieties of cabbage. The difference in pathogenicity or in growth rate was none or only slight, in development of the oospores, however, it was distinct. Three strains were homothallic, forming a great quantity of oospores under suitable conditions. Six behaved unisexually, forming only oospores when grown together with a strain of the opposite thallus group. One strain formed only oospores in a single case, behaving otherwise, as the 6 mentioned above. The 11th strain formed oospores in a small percentage of the tubes when grown either alone or with a strain of the same group, regularly and more abundantly oospores when mixed with a strain of the other group. This strain, therefore, proved to be potentially bisexual, one sex, however, predominating. Of these 8 strains with heterothallic tendencies, 4 belonged to one group and 4 to the opposite group, as proved by intercrossing. As it could not be established in the crosses which mycelium produced the antheridia and which the oogonia, the problem of their sexuality is left undecided.

Damping Off of Alfalfa on Acid and Neutral Iowa Soils. W. F. BUCHHOLTZ.

Pythiaceous fungi have been found especially active on alfalfa seedlings in acid Iowa soils. In 1933 field plots, 41, 48, and 16 per cent were diseased on acid Clarion

loam, Tama silt loam, and Webster silt loam, while on neutral Clarion and Webster silt loam, seven and 6 per cent, respectively, were diseased. In the greenhouse 100 per cent mortality occurred on acid Webster silt loam. The most rapid and general invasion by these soil-borne pathogens has occurred when young emerging seedlings become infected. Lesions on older seedlings were more localized. Steaming or treating with 0.5 per cent formaldehyde either killed or completely inhibited these parasites in acid soil. Limestone, hydrated lime, seed treatment, and soil temperature of 90° C. reduced infection on alfalfa seedlings. Samples of 3 different acid soils were taken at 0 to 2 inches, 2 to 4 inches, 4 to 6 inches, 6 to 8 inches. When planted to alfalfa in the greenhouse 50 seeds per row, the samples in the order named averaged 7, 7, 18, and 39 healthy plants, respectively. Samples of acid Clarion and Tama silt loam, taken in June after two months of dry weather, produced 60 per cent of healthy plants. Greenhouse plantings on similar samples taken in October, after fall rains had kept the soil moist, produced only 12 per cent healthy plants.

Six Microorganisms Pathogenic on Cactus. A. BUZZATI-TRAVERSO.

While a student at the University of Milan, I undertook, in cooperation with Dr. L. Pasinetti, a study of the cactus diseases that occurred spontaneously in 2 collections of 300 species. Three types of symptoms were discerned. A general yellowing, turning to reddish brown with a softening of the tissues developed on plants of *Thelocactus nidulans* and *Cactus maxonii.* Specimens of *Coryphantha valida* and *Neomamillaria Gübrowiana* suddenly developed a wet rot that completely destroyed the plants in a few days. A yellow spotting, followed by wrinkling of the epidermis and a blackening and softening of the tissues, occurred in the cambium in plants of *Cereus peruvianus.* Similar symptoms developed on *Cephalocereus senilis* and *Cereus peruvianus,* except that general necrosis resulted. From the first 5 plants named above, I isolated 5 fungi and from the sixth a bacterium. These were all pathogenic and are as follows: *Fusarium cactacearum, F. cacti maxonii, Sporotrichium cactorium, S. traversianum, Monosporium cactacearum* and *Bacterium cactivorum.* The 2 species of Fusarium and the bacterium are wound parasties, while the two species of Sporotrichium and the Monosporium were able to enter the host in the absence of a wound.

Growth Association of Microorganisms. J. C. CARTER.

Several fungi and bacteria were grown in association. A study was made of the growth reactions, especially the relative inhibition, of the various associations. Only 2 organisms were grown in association, on potato dextrose agar, in a single Petri dish, at any one time. The fungi that were tested were: *Helminthosporium sativum, H. inaequalis, Sclerotinia americana, Glomerella cingulata, Gibberella saubinetti, Fusarium moniliforme, F. conglutinans,* and *F. lycopersici.* Eleven bacterial organisms were used as inhibitors in addition to *Aspergillus niger* and 2 strains of Penicillium. The fungi named above were grown in association with all the inhibitors listed. Of the 14 inhibitors used, *A. niger* is the only one that produced even a slight inhibitory effect on the growth of *F. moniliforme.* The distance that the fungi were inhibited in most cases was from contact to 7 mm. In general, the Fusaria that were tested were not inhibited readily by the organisms with which they were grown in association. Inhibition of *Sclerotinia americana* occurred more frequently at a distance of 5 to 10 mm. While inhibition of *H. inaequalis* generally occurred at 10 to 15 mm. *H. sativum* was inhibited at a distance of 15 to 25 mm. by 3 bacterial inhibitors and *A. niger.* Of the several fungi tested, *H. sativum* demonstrated the most marked inhibition.

Mechanical Transmission of Two Viruses to Pineapple. WALTER CARTER.

Mosaic of *Commelina nudiflora* in Hawaii is apparently identical with that described from Florida. No natural transmission to pineapple is known. Mechanical transmission by needle puncture has been obtained in a low percentage of trials. Initial symptoms develop on the inoculated leaves, below the points of inoculation. The disease is lethal; the symptoms and their development cannot now be differentiated from those of the yellow-spot virus. A single case of needle transmission of yellow spot from pineapple to pineapple showed the same development of initial spots below the points of inoculation, with later development of symptoms typical of field cases.

The Symbionts of Pseudococcus brevipes in Relation to a Phytotoxis Secretion of the Insect. WALTER CARTER.

Green spotting of pineapple leaves is a toxic effect of feeding by *Pseudococcus brevipes.* It is invariably associated with the presence in the insect of a bacillus-like symbiont. When the insect colony is removed from pineapple to Panicum grass, *Panicum barbinode,* the green-spotting capacity is lost. Concurrently, the bacillus-like symbiont disappears. This process is not complete until the second generation of mealy bugs on Panicum grass emerges. In the interim, intermediate stages in the transition of the bacillus can be recognized. The final and apparently stable form is a minute coccus rod, which is found invariably, but often very sparsely, in the non-green spotting mealy bug. Returning the mealy bug colony to pineapple has thus far failed to result in the reappearance of the bacillus or the ability of the mealy bug to produce green spots. It is concluded that the particular effect of the insect's secretion in causing green spotting is due to the presence of this specific symbiont in the bacillus form.

Serological Evidence in the Study of the Relationships of Certain Plant Viruses, K. S. CHESTER.

The precipitin, neutralization, and complement-fixation techniques have been applied to about 40 types and strains of plant viruses. Twenty-five yellow, white, necrotic, symptomless, and ordinary strains of tobacco-mosaic virus all showed such close serological relationship that they may be considered but minor variants of a single type of virus. Aucuba mosaic of tobacco and Johnson's tobacco virus No. 6 also belong within the same serological group. Cucumber mosaic and tobacco ring spot, both entirely distinct from tobacco mosaic, are readily distinguished from each other by the neutralization test, and each of these comprises strains that are serologically very closely related *intra se.* The latent virus of potato differs serologically from all the viruses mentioned above, and is very closely related to potato ring spot, potato mottle, and British Queen streak, the latter 3 behaving as strains of the latent virus. The vein-banding virus of potato, and the aucuba-mosaic virus of potato, are each entirely distinct from one another and from all of the other viruses mentioned above.

The Development of Root-knot Nematode Galls. J. R. CHRISTIE.

The galls studied were caused by the so-called root-knot nematode, *Heterodera marioni,* and produced by experimentally infesting the radicles of tomato seedlings. Penetration takes place near the root tip and, when permanently located, the head of the larval parasite is usually in the plerome with the body extending into the periblem. During the first 48 to 60 hours, cells of the central cylinder, lying in the region of the parasite's head, remain undifferentiated. After 3 to 4 days the walls of these cells disintegrate and the protoplasmic contents of adjacent cells coalesce forming so-called giant cells. In many instances these cells normally would contribute to the formation

of vessels. Tangential division in the pericycle results in the formation of a layer of small, thin-walled, parenchymatous cells not found in normal roots. Secondary roots, frequently present in the region of a gall, are outgrowths of this layer. Eventually, the innermost parenchymatous cells become differentiated into small xylem elements of irregular shape. These abnormal changes apparently are induced by some secretion expelled through the mouth of the nematode and produced by its oesophageal glands. It is of interest to compare these galls with cecidia and with certain pathological conditions in animal tissues.

A New and Important Factor in the Epidemiology of Tobacco Leaf Diseases. E. E. CLAYTON.

The association of wildfire (*B. tabacum*) epidemics with storms has long been known and explained on the basis of dissemination of the organisms. Recent studies indicate that the major storm effect is in breaking down host resistance. Intercellular leaf spaces become flooded during driving rains, resulting in the appearance of well defined water-soaked areas. The bacteria spread rapidly through such areas and necrotic lesions inches in diameter may be formed in 48 hours. Without the aid of water-soaking, *B. tabacum* usually requires a week to produce lesions ¼ inch in diameter. *B. angulatum* attacks the normal leaf with great difficulty and rarely produces lesions larger than ⅛ inch in diameter. With water-soaked tissues, however, this organism quickly produces the large lesions typical of field black fire. In the field, topping and fertilization practices modify the susceptibility of the plant to both wildfire and black fire. These factors modify leaf resistance to water-soaking. Low topping, and low potash or high nitrogen fertilization, increases leaf susceptibility to water-soaking, and hence to disease. High topping, and high potash or low nitrogen fertilization, have the reverse effect. Other results indicate that attack by certain fungus parasites is also greatly facilitated by leaf water-soaking.

Biochemic Studies on the Metabolism of Crown-gall and Hairy-root Bacteria and on the Composition of Crown Galls. H. A. CONNER, A. J. RIKER, and W. H. PETERSON.

Studies of atypical and pathological multiplication of cells are being made through biochemical examinations of bacterial products and of crown galls. Quantitative determinations showed that the crown-gall bacteria grown in an aerated yeast-infusion liquid medium converted about 2 per cent of the glucose fermented to carbon dioxide and about 10 to 15 per cent to bacterial gum. Hairy-root bacteria converted 20 per cent of the glucose fermented to carbon dioxide and 10 per cent to cells and gum. They formed also small amounts of volatile and nonvolatile acids. These were identified, respectively, as acetic by the melting point of its p-toluidide, and as pyruvic by the melting point of its 2–4 dinitrophenylhydrazone. In addition, both organisms produce large amounts of gum-like materials that have been obtained in the form of compounds with heavy metals. Chemical analyses were made of both preserved and fresh galls on tomato and of fresh raspberry galls. Parallel analyses wree made on contiguous healthy tissue for comparison. The galls were higher than the healthy tissue in ash and total nitrogen but lower in cellulose, starch, pentosans, and sugar. No significant differences were noted in the amounts of alpha amino, amide, ammonia, and nitrate nitrogen, or uronic acids.

Occurrence of Oospores of Peronospora effusa with Commercial Spinach Seed. H. T. COOK.

Oospores of *Peronospora effusa,* the fungus causing downy mildew of spinach, have been found mixed with commercial spinach seed in varying quantities during the last

3 years. The seed examined were obtained from 5 commercial seed firms, were of several varieties, and had been grown in different geographical localities. The degree of infestation varied from no oospore to 1 for every 8½ seeds. No correlation was found between the degree of infestation and the variety or geographical locality where grown. The degree of infestation is probably dependent both on the presence of inoculum and on environmental conditions favoring development of mildew. Large numbers of oospores mixed with commercial spinach seed are a potential source of mildew inoculum. They indicate that mildew was severe in the seed crop and that internal infection of part of the seed may have taken place. The crop grown from heavily infested seed in 1932 was badly damaged by mildew, as was the spinach grown from the same lot of seed planted on new land in 1933.

Spinach Seed Treatments in Virginia. H. T. COOK and J. A. CALLENBACH.

Spinach-seed treatments, tested under commercial field conditions during the last 3 years, have resulted in materially increased stands and yields. Copper sulphate solution, and red oxide of copper, zinc oxide, zinc hydroxide, Vasco 4 (a zinc fungicide), and Semesan as dusts were used as treatments. Increases as great as 594 per cent in stand and 42 per cent in yield were obtained. Copper sulphate solution, red oxide of copper, zinc oxide, zinc hydroxide, and Vasco 4 gave approximately equal results, while Semesan treatment was less beneficial. Seed treatment is now practiced on a commercial scale by the local growers and seedsmen, who treat about 90 per cent of the seed used for the early fall crop. Red oxide of copper, which was used at first, has now been replaced to a considerable extent by the zinc materials, which are more uniform physically, more stable chemically, more easily handled, and somewhat lower in cost.

Relation of Insect Injuries and Root Diseases in Sugar Cane. M. T. COOK.

Sugar-cane roots may grow to 6 feet or more in length, but the cortex of all except the last 4 inches dies very early, leading many people to believe that the roots are dead. This dead cortex usually contains many saprophytic fungi. In Puerto Rico three different insects attack sugar-cane roots, the extent of the injuries varying with their location on the roots. It is said by some workers that these injuries become infected by injurious fungi, but the writer's studies indicate that such infection is rare. Some fungi attack the cortex and cause only slight injuries, others are very injurious. Fungi that penetrate into the axis cylinder are most injurious. Thus far the writer has studied the effects of 2 species of Pythium, 1 of Aspergillus, and *Sclerotium rolfsii.*

Relation of Host Vigor to Apple Infection with Xylaria mali. J. S. COOLEY.

Three-year-old apple seedlings, growing at Rosslyn, Virginia, have been inoculated with *Xylaria mali* each month for the past 2 years. The data obtained indicate that susceptibility to infection, size of lesion, and probability of healing are greatly influenced by the state of metabolic activity of the host, which, in turn, is correlated with the season of the year. Evidence indicates that root activity of apple trees varies greatly with the season of the year. The period of greatest susceptibility was associated with the period of least root activity. Suppressed trees, as they grew in the nursery row, were much more susceptible to infection than those growing normally. The fact that suppressed trees showed a much higher percentage of infection during the seasons of slight host susceptibility indicates that the condition of the host as to metabolic activity, rather than other environmental conditions favorable for the fungus, probably is a limiting factor for infection. Trees defoliated in August were much more susceptible to root rot than control trees with leaves. Field observations indicate that a weakened host

condition resulting from drought or other edaphic factors induces greater susceptibility to root-rot infection as it occurs in nature.

Influence of Preceding Crops on Damping Off of Sugar Beets. G. H. COONS and J. E. KOTILA.

Greenhouse experiments with soil from Virginia and Ohio showed that growing sweet clover or alfalfa for 20 to 30 days greatly increased the damping off in sugar-beet plantings that followed. Corn grown for the same period significantly lessened damping off, the basis for comparison in each case being the incidence of damping off in sugar beets occurring in these soils without such preliminary cropping. Beans, as a preceding crop, apparently did not greatly alter initial disease incidence. Using autoclaved soil lightly inoculated (1) with pure cultures of damping-off organisms, or (2) by contamination with unsterilized soil, test beet plantings following sweet clover or alfalfa showed striking increase in damping off; following corn, significant decrease. Such effects did not appear in autoclaved soil that was not inoculated prior to planting preliminary crops. Field evidence has been obtained, in cases where sugar beets immediately followed sweet clover or alfalfa, of the same deleterious influence on stand, in contrast to beneficial effects where corn was the preceding crop. Arrangement of crop rotations to avoid intensification of damping off by alfalfa or the clovers, and to take advantage of its reduction by corn should lead to improved sugar-beet stands.

Some Ecologic Relations of Phytophthora infestans. WILLARD CROSIER and DONALD REDDICK.

The susceptibility of potato plants to attacks of *P. infestans* is not altered by variability of external conditions encountered during the growing period. The most important external factors in the rapid spread of potato late blight are temperature and humidity. A relative humidity of 95 per cent or above must be maintained for about 8 hours to permit the formation of germinable sporangia; and it must persist for a minimum of 11 or 12 hours with liquid water and temperature optima (for germination, swarming, and infection) becoming available at the proper moments in order to permit infection. The spread of late blight over vast areas can not be explained on the basis of aerial migration of the parasite. Infection of new tubers directly from affected seed pieces occurs in sufficient amount to account for the wide prevalence of late blight following a year of no foliage blight.

Scab Resistance in Potato Seedlings. H. M. DARLING, J. G. LEACH, and F. A. KRANTZ.

Of the potato varieties commonly grown in the United States, only those with heavy, russet skins are appreciably resistant to scab (*Actinomyces scabies*). This has led to the popular assumption that resistance to scab is correlated with thick or russet skin. During the past 3 years a large number of seedlings of inbred lines of varied origin have been tested for scab-resistance on heavily infested soil. There is a wide range of resistance among the seedlings tested. A high degree of resistance was found in smooth- and thin-skin seedlings, as well as in the russet types. There was no correlation between color of tuber and scab resistance. Histological studies indicate that resistance is closely associated with the rapidity and efficiency of the formation of normal and wound cork in the lenticels and with the type of cells making up the lenticel structure. Those seedlings having lenticels with smaller and more compact cork cells appear to be more resistant. When certain susceptible seedlings were inbred, both susceptible and resistant seedlings were obtained in the progeny. Likewise, when resistant seedlings were inbred, both susceptible and resistant seedlings were obtained in the progeny. Certain resistant

seedlings, however, yielded seedlings, almost all of which were resistant. A few were slightly more susceptible than the parent. (Divisions of Plant Pathology and Horticulture of the Minnesota Agricultural Experiment Station and the Office of Horticultural Crops and Diseases of the United States Department of Agriculture, cooperating.)

Mycorrhizal and Pseudomycorrhizal Infections of Pine Roots During First Year's Growth. K. D. DOAK and P. L. FISHER.

Determination of the relations involving mycorrhizae in 1-year pine without dissection requires accounting for each portion of the root system and the infections present. Mycorrhizae are distinguished from pseudomycorrhizae by the external mantle that covers the tips, and shows a distinct line of demarcation where it terminates near the mother root. Hypertrophy is evident in true mycorrhizae but lacking or very slight in pseudomycorrhizae. Root hairs are absent from both except for occasional appearances on the noninfected basal portion of the short root. Dichotomy is more frequent in mycorrhizae than in pseudomycorrhizae, even though the latter show slow continued elongation. Rarely short roots show development with both mycorrhizal and pseudomycorrhizal characters; but, when this occurs, each part is separate and distinguishable. Dead mycorrhizae may appear late during the first year's growth, but they are distinguished easily from living mycorrhizae and pseudomycorrhizae. In nurseries of the Allegheny Region, *Pinus strobus, P. taeda, P. echinata* and *P. resinosa* show development of pseudomycorrhizal, as well as mycorrhizal, short roots during first year's growth, but the proportion varies with the nursery. Analyses indicate that the number of each of them is greater than that of noninfected short roots, which appear infrequently.

A Pythium Species of the Megalacanthum Type in Cineraria Roots and the Relation of Putrefaction to Parasitism Among the Pythiaceae. CHARLES DRECHSLER.

Among the Pythiaceae, incapacity for mycelial growth in presence of bacterial contamination necessitates parasitism in living plant structures, or saprophytic invasion of organic materials in advance of competing fungi and attendant bacteria. Such invasion requires rapid mycelial extension. Comparatively slow-growing forms, adversely affected by bacteria, as, *e.g.*, species of Phytophthora generally, are, therefore, constrained in nature to operate mostly as obligate parasites. Rapidly growing forms, similarly affected, including the more familiar species of Pythium, may, however, live also semiparasitically on moribund plant tissues, or saprophytically on tissues newly dead. A few Pythium species—the anomalous *P. anandrum,* and especially *P. mastophorum* and *P. polymastum,* both closely related to *P. megalacanthum*—show greater resistance toward bacteria, producing zoospores in moderately foul water. Large oogonia with basally thick-walled, somewhat mastoid protuberances, characteristic of the *megalacanthum* series, occur in roots of herbaceous plants somewhat more frequently than might be inferred from the paucity of relevant records. Such oogonia were especially abundant in discolored roots of potted Cineraria plants obtained from a greenhouse near Alexandria, Va., April, 1933. The oospores, though normal, failed to germinate, and the fungus was not isolated.

Occurrence of a Species of Aphanomyces on Roots of Spinach and Flax. CHARLES DRECHSLER.

From the discolored roots of somewhat stunted spinach plants collected near Norfolk, Va., Nov. 25, 1932, as also from affected roots of spinach collected in New Jersey, May, 1930, were isolated, in addition to various species of Pythium, many cultures of a species of Aphanomyces. This species has nearly the same dimensions as have been

published for *Aphanomyces cladogamus*. As in the latter, too, the oogonial stalk and the antheridial branch frequently arise close together from the same hypha; and the oogonial stalk rather regularly passes spirally around the antheridial branch. The fungus is, therefore, probably identical with *A. cladogamus;* in any case, it certainly differs from *A. euteiches*. Apparently the same species of Aphanomyces was obtained in numerous cultures derived from discolored roots of stunted flax plants collected in an excessively wet field near Butternut, Wis., July 10, 1931. The discoloration, mostly yellowish and orange, occurred in specks and patches on the surface of the main root. Though the affected portions of the cortex were noticeably softer than the healthy portions, no general decay of the root was present at the time of collection.

Thermal Inactivation of Some Tobacco Viruses: Standardization, Technique, and Illustrative Data. B. M. DUGGAR.

The temperature lag involved in the determination of thermal inactivation periods was followed with extracted juices of diseased plants, using as containers for the extracts test tubes of medium (culture) and small (serological) size in contrast with standardized, thin-walled, capillary glass tubes. In addition to the thermal bath the apparatus consists of a thermocouple in conjunction with a potentiometer and a galvanometer setup, also suitable stirring devices in the case of the tubes. Readings were made every 15 seconds. A significant lag period was found with the tube methods but no measurable lag with the capillary methods. Accordingly, the capillary method is recommended as a standard technique in precise work on thermal inactivation. Data are presented showing the inactivation percentages or survivor values for the virus of typical tobacco mosaics and for the virus of tobacco ring spot. A direct leaf-blade-immersion method also has been used, and with these viruses the technique is comparable to the capillary method. Owing to differences in tissue texture in other host plants, the tissue method is not regarded as having such general applicability.

Some Factors Affecting "Longevity" in Vitro of Viruses of Tobacco Mosaic and of Tobacco Ring Spot. B. M. DUGGAR and D. F. MCALISTER.

The survivor value of the virus of typical tobacco mosaic in tobacco extract is favorably affected (1) by condensation of the extract at low pressure and temperature; likewise (2) by the addition of low concentrations (antiseptic) of ethyl alcohol. The active period of the virus of tobacco ring spot (Wingard strain) also is prolonged, within limits, by low concentration of alcohol and by the addition of certain buffers at definite pH levels, notably around pH 5.8–7.0, phosphate buffers being more favorable than citrate. The dispersion of the ring-spot virus also is enhanced by the phosphate buffer, as measured by incidence of primary infection lesions.

Sand Culture of Seedlings as a Damping-off Control. A. A. DUNLAP.

Certain species of seedlings that have shown tendencies toward damping-off in soil have been grown successfully in pure sand, with mineral nutrients added in solution. Both preemergence and postemergence damping-off have been much less in the sand than in nontreated soil. The results of growing seedlings in sand, have compared favorably with those obtained from the use of the usual seed or soil treatments.

A Neglected Factor in the Planning and Interpretation of Fungicidal Tests. H. W. DYE.

Practically all comparative fungicidal tests on apples are applied according to a schedule developed over a period of years through the use of lime-sulphur solution, and

are based on the minimum number of applications with a material that, because of its nature, retards normal development to a varying extent. In comparative tests with fungicides that allow a more normal growth, the advantage in control is always with the material that permits the least foliage and fruit development between applications, even though all may be of equal fungicidal value. Is it not possible that this simple difference in the growth factor with different fungicides, which is often overlooked, may explain some of the differences that occur in comparative fungicidal tests, and is it not worthy of more serious consideration in planning such tests and in interpreting their results?

Soil Treatment with Sulphur and Limestone for Control of Bacterial Wilt of Potatoes. A. H. EDDINS.

Inoculated sulphur applied to the soil at the rates of 200, 400, 600, 800 and 1,200 pounds per acre decreased the percentage of bacterial wilt (*Bacterium solanacearum*) infection of potatoes in Florida. Control was correlated with the amount of sulphur applied, as the disease disappeared most rapidly and most completely from plots receiving the greatest amounts where the pH of the soil was reduced to 4.50 and lower. During 5 seasons the most beneficial rate at LaCrosse, Florida, was 800 pounds per acre and during 2 seasons at West Tocoi, 600 pounds per acre. Smaller quantities were less effective, while larger applications reduced the yields of potatoes. Agricultural limestone applied in January, 1934, at LaCrosse, at the rate of 2,000 pounds per acre to plots treated with 1,200 pounds of inoculated sulphur per acre in 1929 increased the yield of healthy marketable tubers 12.7 barrels per acre, as compared to the yield of plots receiving neither of these agents; tuber infection in the treated plots was 0.3 per cent and in the check plots 2.6 per cent. A like amount of limestone applied in November, 1933, at West Tocoi, to plots treated with 800 pounds of inoculated sulphur per acre in October, 1932, increased the yield 17.1 barrels per acre; tuber infection in the treated soil was 0.4 per cent and in untreated soil 25.7 per cent.

Field Trials of Pentachlorethane, Tetrachlorethane, and Xylol as Affecting Phymatotrichum Root Rot and Host Plants. WALTER N. EZEKIEL and J. J. TAUBENHAUS.

Pentachlorethane, tetrachlorethane, and industrial xylol were applied in the soil around cotton plants and chinaberry, maple, and elm trees with *Phymatotrichum omnivorum* root rot, in holes 6 × 6 inches apart and 6 inches deep, at rates calculated on the soil weight usually to 4 feet deep. Roots were excavated and cultured to determine viability of the fungus. At 500 and 1,000 ppm., the materials killed *P. omnivorum* on and within these roots; 250 ppm. was not uniformly effective. Whether the fungus is completely eradicated from the soil in treated areas remains to be determined. Applied around normal trees or shrubs left undisturbed after treatment, tetrachlorethane at 500 or 1,000 ppm. caused rapid, severe injury to these plants, xylol slower injury, and pentachlorethane still slower and less severe injury. In some other plats, materials were applied to the soil at a higher rate prior to planting cotton. Only scattered stunted plants were obtained from plantings made even 3 months after the tetrachlorethane treatment; while a good stand was secured from a planting 16 days after the xylol treatment.

Infection of Apple Leaves by Physalospora cydoniae. H. H. FOSTER.

Leaves of potted apple trees were inoculated by spraying with pycnospores of 30 isolates from different regions. Following inoculation, the trees were held in a moist chamber for 24 hours at 20° C., and incubated in the greenhouse. Only 10 of the 30

isolates gave consistent infection. The fungus was reisolated at will. Eight of the 10 leaf-infecting isolates were secured from rotted apple and quince fruits from eastern States, one was obtained from a Chinese quince fruit from France, and one from an apple leaf from South Carolina. Isolates obtained from Wisconsin and certain nighboring States induced little or no infection. Some isolates induced visible lesions within 24 hours; others only after 48 to 72 hours. Penetration was found to take place through the stomata. In 2 trials 21 varieties of apple trees were tested for comparative susceptibility of leaves to *Physalospora cydoniae*. Two isolates, each inducing infection, but differing in certain respects, were used in these trials. Some indication of differences in varietal susceptibility was apparent.

Spotted Wilt of Truck Crops and Ornamental Plants. M. W. GARDNER, C. M. TOMKINS, and O. C. WHIPPLE.

The host range of the virus of the spotted wilt of tomatoes in California, as indicated by inoculation with the virus in the green house or recovery of the virus from naturally infected plants, includes, in addition to the hosts reported in previous abstracts, cauliflower, celery, *Nicotiana glauca*, and species of Amaryllis, Begonia, Browallia, Campanula, Cheiranthus, Delphinium, Emilia, Gloxinia, Godetia, Gaillardia, Layia, Papaver, Pentstemon, Primula, Salvia, and Verbena. The virus has been transmitted with *Thrips tabaci* from Emilia to Emilia and tomato, and from calla to tomato, and with *Frankliniella* sp. from Datura to poppy and from tomato to tobacco. The virus has been found to survive 73 hours' storage at 0° C. The disease is most prevalent and severe in the cooler coastal districts, particularly where truck crops or ornamentals are grown more or less all the year round. Relatively low temperatures seem to favor the disease.

Availability of Copper in Bordeaux Mixture Residues and Its Absorption by Conidia of Sclerotinia fructicola. M. C. GOLDSWORTHY and E. L. GREEN.

The available copper in 2–4–50 Bordeaux-mixture residues that had been subjected to aging and weathering and that absorbed within the conidia and germ tubes of *S. fructicola* were determined by the use of nitroso-chromotropic acid. The available copper, inherent in such residues and amounting to at least four ppm. by volume of the original mixture, was lethal to germinating and germinated conidia. Smaller amounts of available copper were found to be inhibitory to the growth of the conidia. With inactive conidia, intake of copper was relatively slow, but with actively germinated conidia, it was very rapid. The intake of available copper was not affected by conidial secretions or metabolic by-products. Intake of copper was associated with intake of other ions. Germinating conidia do not have the power to restore the supply from the remaining insoluble residue, once the available copper is washed away by rains. Restoration of the available copper is apparently brought about by weathering.

Control of Aster Leaf Rust. C. E. F. GUTERMAN.

Leaf rust (*Coleosporium solidaginis*) is very prevalent in commercial plantings of the China aster in New York State. During the 1934 growing season, several sprays and dusts were tested under field conditions in an attempt to obtain control of the disease. The following fungicides, listed in the order of their efficiency, were used: Sulfodust, Green Kolodust, Flotation sulphur spray and 3–1½–50 Bordeaux mixture. Due to abundant rainfall and an unusual amount of rust, 8 applications were necessary to provide adequate protection. With the check plots averaging 18.9 lesions per leaf, a reduction to 0.2 lesions per leaf was obtained with the Sulfodust. Marked differences in

varietal susceptibility to rust were observed in tests involving 60 commercial varieties of the China aster. Under the conditions of these experiments, the varieties California Giant, white; Giant Branching Comet; Salmon Queen; Imbricated Pompon, blood red; Lilliput flowered, rose; Lilliput flowered, dark blue; and Lilliput flowered, white, proved to be essentially immune.

A Liquid Formaldehyde Treatment to Control Damping Off of Flower Seedlings. C. E. F. GUTERMAN and L. M. MASSEY.

Six per cent formaldehyde dust used at the rate of 1½ oz. per square foot for the average-size flat is a common method now in use for damping-off control. When used in proper amounts, a 40 per cent formaldehyde solution has given results equally as good and often better. Using a soil mixture consisting of half top soil and half sand in flats measuring $18\frac{1}{2} \times 14 \times 3$ inches, it has been determined that $12\frac{3}{4}$ cc. of the formaldehyde solution when diluted 5 or 6 times its volume with water and thoroughly mixed with the soil will give excellent results. At this streigth, a 12–24 hour wait is necessary after the treatment before it is safe to plant most seeds. Six to 9 cc. may be used for the same amount of soil and the seeds planted at once. As is true with formaldehyde dust, the flats must be watered thoroughly after sowing the seed. Among the ornamentals that respond well to this treatment are Calendula, China aster, Clarkia, Gypsophila, Kochia, larkspur, Lunaria, marigold, Scabiosa, stock, strawflower, and sweet pea. Anchusa, Campanula, and snapdragon gave irregular results due to injury. The advantages of the method are its ease of application, economy, and assurance of uniform strength.

The Effect of Mosaic on Transpiration of the Bean. A. L. HARRISON.

The effect of mosaic on transpiration of Stringless Green Pod Refugee beans, *Phaseolus vulgaris*, was determined by several methods; namely, auto-irrigators (Livingston and porous-pot), balances, and cobalt chloride papers. The rate of transpiration was significantly less per unit of surface area and per unit of dry weight in mosaic than in healthy plants. Leaves with primary symptoms transpire much slower than leaves with secondary symptoms, as measured by cobalt chloride papers. The same method revealed that the light green areas of mosaic leaves transpired significantly less than the dark green areas. Mosaic did not effect the rate of transpiration on leaves formed before infection. Leaf areas were determined by multiplying the product of the width and length of the leaves by the conversion factor for the leaf or leaflet in question. Detached pods from mosaic plants transpire faster per unit of surface area and per unit of fresh weight than detached pods of the same size from healthy plants. Size of pod has a greater influence than age on the rate of transpiration. Small pods transpire faster per unit of surface area and per unit of fresh weight than large pods of the same age. (Cooperative investigation between the New York (Geneva) and the Cornell University Agricultural Experiment Stations.)

Vascular Disease in Poplar and Willow. CARL HARTLEY and B. S. CRANDALL.

There occurs in the District of Columbia a stain in the wood of Lombardy poplar, *Populus nigra italica*, at first water-soaked but later changing to brown, commonly with an intermediate red stage. It was noted by L. W. R. Jackson some years ago in dying trees, and is now found to be of very common occurrence in apparently healthy trees. It does not cause death until it has occupied almost the entire cross section of the trunk. Short rod bacteria, forming quantities of gas, are readily isolated from wood near the margin of the discoloration. Observation indicates this vascular disease to be one of the

principal causes of the short life of the Lombardy poplar in the neighborhood of Washington. A somewhat similar condition is frequent in cottonwood, *P. deltoides;* goat willow, *Salix caprea;* and some other willows not yet identified. In both poplar and willow the symptoms in some respects suggest the water-mark disease of willow in Europe. Reddish discolorations containing bacteria also occur in numerous young plane trees, *Platanus* sp., in a nursery near Washington. (Bureau of Plant Industry and Emergency Conservation Work.)

Control of Downy Mildew of Tobacco. R. G. HENDERSON.

A number of fungicides have been tested under greenhouse conditions for the control of downy mildew of tobacco. Some of the materials included in these experiments are: Benzoic acid, cuprous oxide, coposil, copper phosphate, acetic acid, and copper-molasses mixture. Of these, benzoic acid, cuprous oxide, and copper-molasses mixture were the most effective. Cuprous oxide delayed the development of the disease several days, but under conditions most favorable for the disease, this spray did not prevent it. Benzoic acid was more effective than cuprous oxide and should be tested further. The copper-molasses mixture was very effective in controlling mildew, although, in these tests some mildew developed on seedlings sprayed with this material. As reported last year, calcium sulphide has given very good control of downy mildew. Under greenhouse conditions the growth of plants sprayed with calcium sulphide was very much retarded, but in experiments conducted last spring in outside beds the plants did not show this stunting effect. In the greenhouse experiments, calcium sulphide applied at 3-day intervals gave better control of mildew than the copper-molasses mixture. Several other chemicals in addition to those mentioned above are now being tested.

Studies on the Soft Rot and Colon-Typhoid-Dysentery Groups of Bacteria. 1. Pathogenicity. W. D. HENRY.

One hundred and twenty organisms belonging to either the soft-rot or colon-typhoid-dysentery group of bacteria have been used in repeated attempts to study the variability in pathogenicity of these organisms. Carrot varieties, Oxheart, Chantenay, Long Orange, and Danvers Half Long were used as the chief test host but one or more tests were made on cabbage, potato, iris bulbs, delphinium, radish, turnip, lettuce, banana, parsnip, and canna. Aseptically cut slices from sterilized carrots were placed in sterile moist Petri dishes. Inoculation was made by placing one loopful of culture in Bacto-beef broth on the freshly cut surface of carrot slices. Results obtained showed considerable differences in pathogenicity of organisms causing rot on carrots of different varieties and also on carrots of the same variety. Sixty-six out of these 120 organisms rotted Oxheart variety carrots. However, not all of these proved consistently pathogenic. The first series of inoculations showed that 47 organisms caused rot, 46 proved pathogenic in the second trial, only 24 in the third and 17 in the fourth. The inoculation experiments extended over 10 months. Some organisms retained their pathogenicity in all tests, while others lost and regained it without any regularity. Additional trials with other varieties of carrots gave similar results.

Apothecium Production in Sclerotinia trifoliorum and S. sclerotiorum. LAWRENCE HENSON.

In a study of crown rot of red clover at Lexington, Kentucky, apothecia of two species of Sclerotinia were found in large numbers—one (*S. trifoliorum*) in October, the other (*S. sclerotiorum*) in May. A satisfactory technique for production of apothecia consists in planting sclerotia on plain 1 per cent agar slants in 2×8.5 cm. vials plugged

with cotton. Apothecia developed in vials out of doors simultaneously with those of the same strains planted in soil. *S. sclerotiorum* developed stipes in the icebox at 48 to 51° F. in 50 to 60 days, and *S. trifoliorum* in 25 to 30 days. When transferred to light, both species matured normal apothecia. Of 63 cultures of *S. trifoliorum* placed out of doors in the fall, 51 matured apothecia; but 17 cultures of *S. sclerotiorum*, set out on September 27, failed to do so. In day-to-day plantings out of doors, some stipes developed in 14 days, the average temperature being 64.5° F. The production of functional apothecia by 34 single-spore cultures of *S. trifoliorum* from 7 apothecia, and by 3 single-spore cultures of *S. sclerotiorum*, 9 single-spore cultures of which again developed functional apothecia, demonstrates that at least some strains of these species are homothallic. (Cooperative investigations betweeen the Division of Forage Crops and Diseases, Bureau of Plant Industry, Department of Agriculture, and the Kentucky Agricultural Experiment Station.)

Longevity of the Fire-blight Organism in the Honeybee Environment. E. M. HILDEBRAND.

The longevity of the fire-blight organism, *Erwinia amylovora*, when introduced into the honeybee environment has an important bearing on the question of whether the honeybee is capable of overwintering the bacteria. Studies conducted in New York over a period of 3 years show that the probability of the bacteria overwintering in the beehive, when introduced through the normal channel of food, is remote, because of: (1) The disappearance within 3 days of the bacteria from honey elaborated from food containing the organisms; (2) the inability to identify the organism with stages of the life cycle of the insect; (3) the relatively high temperatures (up to 35° C.) of the bodies of honeybees and of the interiors of hives; (4) the shortness of life of the bacteria held in sugar solutions at different concentrations and constant temperatures; and (5) the relatively poor dissemination of the bacteria to flowers when caged with honeybees that had been fed heavily infested sugar solutions. The same colonies of bees were used throughout the series of tests without apparent injury. The validity of the conclusions of these studies, a detailed report of which will be published, receive support from studies conducted elsewhere on the shortness of life of other bacteria in honey.

Modes of Entry of Erwinia amylovora into the Flowers of the Principal Pome Fruits. E. M. HILDEBRAND and L. H. MACDANIELS.

Blossom-blight control with bactericides has been attempted, as blossom time is a critical period in the life history of the fire-blight organism. The results of studies begun in 1932 were variable and sectioning of the flowers revealed a different picture from that in the literature with respect to the natural openings. In view of this situation the need was apparent for an understanding of the normal and pathological anatomy of the flowers, and such studies were undertaken with station coworkers. The bacteria apparently enter the flowers of pear, apple, and quince only through natural, or artificial, openings. The types of natural openings encountered were: (1) Noncutinized tissue (stigmatic surface, anther); (2) hydathodes (sepals); (3) stomata (style, fruit exterior, sepals); and (4) specialized nectar-secreting stomata (receptacle cup). The distribution of the natural openings was such that they functioned readily as infection courts to the bacteria, whether the agency of dissemination was rain or insects. The floral organs varied in their relative susceptibility to invasion. When atomized with bacterial suspensions, the receptacle cup of pear and the stigmas of apple and quince were frequently invaded. The remoter avenues of entry, as petals and anthers, sometimes resulted in blossom infection when they alone were inoculated.

Some Fungicidal Possibilities of Red Copper Oxide. JAMES G. HORSFALL and JAMES M. HAMILTON.

This is a progress report dealing with fungicidal qualities of red copper (cuprous) oxide, based upon data obtained, using the material as a seed disinfectant and protectant and as a foliage spray and dust, in comparison with standard fungicides. (1) It gave satisfactory control of seed decay, oat smut, wheat bunt, apple scab, tomato early blight, rose mildew and black spot, and snowberry anthracnose. (2) It sometimes gave typical injury on copper-sensitive plants, but seemed as generally safe as Bordeaux. (3) Lime and cottonseed-oil emulsion reduced the injury without curtailing effectiveness. (4) It did not stunt hop cones nor distort succulent tomato and rose leaves when Bordeaux did. (5) It adhered strongly to seeds and foliage and persisted through rains because it is highly insoluble, has a small particle size, and probably bears a positive electric charge. (6) The high weight/volume ratio and small particle size suggest that it can be dusted with greater than usual velocity. (7) High copper content of the molecule almost eliminated objectionable spray residue. (8) The cuprous ion purchasable cheaply in cuprous oxide seemed more toxic than the cupric ion widely used in plant pathology. (9) It seems compatible with usual insecticides.

Inoculation Tests with Phytomonas stewarti and P. vasculara. S. S. IVANOFF.

Greenhouse and field inoculations on sorghum, *Holcus sorghum,* Sudan grass, *Holcus sudanensis,* and yellow foxtail, *Setaria glauca,* with strains of *Phytomonas stewarti* induced symptoms resembling those caused by the same pathogen on corn. The degree of similarity depended on the host. Young sorghum plants in the greenhouse, inoculated at the base by punctures, developed red stripes along the leaf veins. The shape and development of these stripes resembled those induced on corn. Field inoculations on sorghum induced reddening and plugging of the vascular bundles, with occasional formation of pith cavities. Red leaf stripes, similar to those produced on sorghum in the greenhouse, were produced on Sudan grass in the field. The stripes occurring on the leaves of yellow foxtail were narrow and tan colored. In the greenhouse, sweet corn plants, when inoculated at the base with *P. vasculara,* developed symptoms similar to those induced in the same host by *P. stewarti,* such as water-soaked stripes upon the leaves, stalk rot, and general stunting. Puncture inoculations of sorghum plants with the sugar-cane organism induced leaf symptoms resembling those induced by the same pathogen on sugar cane, *Saccharum officinarum,* and by *P. stewarti* on sorghum.

Resistance of Sweet Corn to Bacterial Wilt. S. S. IVANOFF and A. J. RIKER.

Studies on resistance of sweet corn to bacterial wilt (*Phytomonas stewarti*) were conducted during the summers of 1933 and 1934. Resistance was tested both by artificially induced and natural infection of more than 200,000 plants, including approximately 2,000 inbreds and hybrids secured from Golden Bantam. The size of the experiment and the uniformity and efficiency of introducing 5 wilt-inducing strains of the organism into the plants was greatly increased by using a plant inoculator. Although no immune plants were found, considerable differences in susceptibility were observed among the strains. In the same inbred strain, acquired vigor, because of better growing conditions, appeared directly correlated with increased resistance. Apparently, hybrid vigor is responsible for some resistance. At least 3 other factors commonly appeared correlated with resistance: inherent vigor, time of maturation, and "true resistance." Usually, late strains were more resistant than early ones. True resistance was found in vigorous as well as in weak, in late as well as in early strains. After consideration of factors mentioned above, it appeared commonly that crosses between susceptible and

susceptible inbreds gave susceptible hybrids; between susceptible and resistant inbreds gave resistant hybrids; and between resistant and resistant inbreds gave resistant hybrids.

A Phytophthora Root and Collar Rot of Pinus resinosa Seedlings. L. W. R. JACKSON and B. S. CRANDALL.

The red pine, *Pinus resinosa,* seedling and transplant stock in one of the eastern forest nurseries has suffered heavy losses for several years from a detructive type of root and collar rot. The disease is characterized by a dry type of bark rot and a vascular infection that causes the wood to become dark colored and very resinous. Large pitch pockets in the wood are common. The infection involves the entire root system and may extend upward several inches in the stem. Isolations from root and collar infections consistently yielded a fairly high percentage of cultures of a Phytophthora closely resembling *P. cinnamomi.* In artificial inoculations of potted red pine plants, the fungus produced disease symptoms similar to those occurring in nature. By means of various kinds of artificial inoculations the fungus has been found to be the causal organism of the root and collar rot of red pine. Other fungi found associated with the root and collar rot lesions were *Pestalozzia* sp., *Sphaeropsis* sp., *Sclerotium* sp., and *Rhizoctonia* sp. All of these fungi when tried in artificial inoculations were capable of causing resiniferous lesions, and sometimes death, but failed to reproduce the typical symptoms of root and collar rot. (Bureau of Plant Industry and Emergency Conservation Work.)

A New Disease Affecting Platanus orientalis in the Eastern United States. L. W. R. JACKSON and BAILEY SLEETH.

Oriental planes in one community in Delaware County, Pa., have been dying at an alarming rate for several years. Within a year or two the foliage of the afflicted tree becomes exceedingly sparse and then suddenly wilts. The bark on the main trunk is covered with numerous longitudinal lesions, which are associated with black wood discolorations that may extend inward to the pith. Isolations from the discolored wood have consistently yielded pure cultures of an endoconidiophora form of Ceratostomella. In culture it gives off a strong banana-oil odor. Fourteen 2-year-old cuttings of oriental plane, in greenhouse pots, were inoculated and all of them wilted after 4 to 13 weeks. Wilting occurred only on parts above the point of inoculation. The fungus produced a black vascular discoloration in the stem of the inoculated cuttings, for a distance up to 6 inches, which was identical with the infections in nature. The reisolations yielded pure cultures of the fungus in almost every case. Inoculations without wounding produced, in 11 days, bark lesions ½ inch long. Inoculations on 2-year-old seedlings of *Ulmus americana, Quercus palustris,* and *Q. borealis* failed to produce infections. No inoculations were made on *Platanus occidentalis.* (Bureau of Plant Industry and Emergency Conservation Work.)

The Value of Zinc Sulphate as a Peach Spray Ingredient. K. J. KADOW and H. W. ANDERSON.

Experiments have been conducted in Illinois (1929 to 1934) to determine, if possible, the value of zinc sulphate as a fungicide and bactericide, as well as its relation to peach growth and spray injury. The results indicate that zinc sulphate added to lime or lead arsenate-lime spray does not improve their fungicidal or bactericidal value. Lime alone is more effective than zinc sulphate. Zinc sulphate improved the growth of peach seedlings grown in sand and maintained by a nutrient solution, but had no noticeable

effect on seedlings grown in black silt-loam. No growth improvement has been observed when zinc sulphate-lime or zinc sulphate-lead-lime sprays were applied to the peach under Illinois field conditions. Field records and chemical analyses show conclusively that zinc sulphate added to lead arsenate-lime sprays almost eliminates water-soluble arsenic from the mixture. The chemistry of this reaction is not fully understood. Apparently its action may be two-fold: the prevention of rapid carbonation of lime and precipitation of water-soluble arsenic as zinc arsenate whenever it is formed. When lead arsenate-lime sprays are applied to the peach in Illinois zinc should always be added to prevent spray injury. Zinc sulphate has no commercial value when applied specifically as a fungicide, bactericide, or plant stimulant.

The Production of Asexual Spores by Pleurotus corticatus. FRANK KAUFERT.

In 1931 an agaric, tentatively identified as *Pleurotus corticatus,* was isolated from fire-scarred and decayed red gum and oak in the Mississippi Delta of Louisiana and Mississippi. Large, black-top coremia developed on the pileus, gills, and stipe of a fruiting body found in nature. They were found also on mycelium in culture and on fruiting bodies produced in pure culture, when placed in a moist chamber. Binucleate oidia are borne in a drop of colorless liquid on the ends of the compact coremia. In addition, round, binucleate conidia are borne singly on tips of hyphal branches of the mycelium. On haploid mycelia, grown from single basidiospores, coremia, and conidia are formed also, but are smaller than the binucleate ones.

Progress in the Development of Eradicant Fungicides. G. W. KEITT.

Many plant diseases are combated chiefly by repeated applications of *protectant* fungicides. Usually enough infection occurs to occasion annually recurring primary inocula, often abundant and difficult to control. Studies here reported aim at developing *eradicant* fungicides adapted for direct attack upon pathogens at vulnerable stages. Strong eradicant fungicidal properties have been demonstrated for various mixtures of copper sulphate, lime, and certain arsenites (notably of calcium, copper, zinc, and iron) in water. Such mixtures release toxic materials over a long period. Early results from their use against *Venturia inaequalis* were reported to this society. Later results against several pathogens, mostly from small-scale experiments, follow: Spraying leaves in fall prevented or sharply limited ascospore production by *Ventura inaequalis, V. pyrina,* and *Coccomyces hiemalis.* Some preparations caused serious host injury: others did not. Late dormant spraying killed the perithecia of *Plowrightia morbosa* and *Mycosphaerella rubina,* and prevented or sharply limited production of conidia by *Sclerotinia fructicola.* High effectiveness against fruiting structures at or very near the surfaces of invaded tissues is demonstrated. Potential vulnerability of many pathogens to attack by surface applications of suitable eradicant fungicides is indicated. This report is made to invite efforts to improve and practically adapt eradicant fungicides.

Fungicidal Properties of Certain Copper-lime-arsenite Preparations. G. W. KEITT and D. H. PALMITER.

Toxicities of various preparations of copper sulphate, lime, and certain arsenites (especially of calcium) in water have been studied by a standardized cultural technique in which plant pathogens were incubated on plates of malt agar containing the toxic materials. Mono-, di-, and tricalcium arsenite, each used alone, equaled or exceeded copper sulphate in toxicity. The toxicity of copper sulphate-lime-calcium arsenite preparations equaled or exceeded the additive toxicities of the copper sulphate and calcium arsenite used in them. Such preparations showed high toxicity to all of 10 plant pathogens

tested. Copper-lime-calcium arsenite preparations showed powerful fungicidal action by materials that diffused through the agar. This was strongest in initially acid media, but also strong in media initially moderately alkaline. Each calcium arsenite, used alone, showed similar but less powerful fungicidal action. The copper-lime component, used alone, showed no perceptible fungicidal action by diffused compounds in alkaline media and very little in neutral or slightly acid media. The fungicidal properties of copper-lime-arsenite preparations vary greatly with modifications in relative amounts of the component materials and with the choice of arsenites, which have a wide range of solubility and other important properties.

Pathologic Significance of Seed-Coat Injury in Dent Corn. BENJAMIN KOEHLER.

A slight puncture in the seed coat at the crown caused a 12 to 16 per cent loss in yield of grain, while removal of the seed coat from the whole crown resulted in a loss of 18 to 23 per cent. A cut through the seed coat at the side of the kernel where it is underlain with horny endosperm caused no injury to stand or yield. Breaking away of the tip cap where the kernel is attached to the cap caused no apparent injury. Inoculation experiments in the field showed that, under wet soil conditions, *Aspergillus flavus* and *A. tamarii* caused marked injury to corn with seed coat injured at the crown, producing virescent seedlings, many of which died from lack of chlorophyll. Injury from *Gibberella saubinetii* was confined to dry soil conditions and was most pronounced in plots planted with seed injured at the crown. *Penicillium oxalicum,* also, was much more active on grains injured at the crown than on sound grains, under both wet and dry soil conditions. A seed treatment with ethyl mercury phosphate practically prevented all loss to stand or yield resulting from injured seed coats.

Heat Treatment for the Cure of Yellows and Rosette of Peach. L. O. KUNKEL.

Peach trees infected with either peach yellows or peach rosette virus may be cured by holding them at high temperatures. Cured trees remain healthy in appearance indefinitely, and graft inoculations from them to healthy trees do not cause disease. Experimental cures were obtained by incubating potted trees in a hot room at a temperature varying from 34.4° to 36.3° C. for 2 weeks or longer. Shorter periods of treatment were not uniformly effective. Exposures up to 3 weeks caused no serious injury to the trees. Since moderately high temperatures destroy these viruses, it is believed that the southern distribution of yellows and rosette may be determined by summer temperatures. Some difficulty was encountered in destroying the viruses in roots. The roots are embedded in earth that must be kept moist. They do not, on account of evaporation, reach the temperature of the tops. Retention of these viruses by rooots of treated trees suggests that the virus of phony disease may be confined to roots because of the high summer temperatures to which aboveground portions of trees are exposed in areas where this disease occurs.

A Phytophthora Wilt of Black-Locust Seedlings. E. B. LAMBERT and B. S. CRANDALL.

A wilt of black-locust seedlings caused considerable loss in 1933 and 1934 in a Virginia nursery. Consistent isolation, inoculation, and reisolation experiments proved that the disease is caused by a species of Phytophthora, which apparently can enter young and tender plant parts only. During the first 3 weeks after a seedling emerges the entire attacked plant wilts; but if it escapes until it is a month or more old, usually only the new growth wilts and the plant recovers. Drooping of the cotyledons, usually the first symptom, is rapidly followed by the collapse and shriveling of the entire seedling. The white swollen bases of the stems and the roots are the last to be affected, in com-

plete contrast with the damping-off symptoms that result from Rhizoctonia attack on the same host. In field experiments in 1934, the Phytophthora wilt was effectively controlled by scarifying the seed and spraying with Bordeaux mixture to protect the emerging seedlings and new growth. Four applications were made at the rate of 6 gallons per 400 square feet. The incidence of the disease was also greatly reduced by increasing the acidity of the upper 3 inches of soil from pH 6.2 to 4.6 with aluminum sulphate. (Bureau of Plant Industry and Emergency Conservation Work.)

Resistance to Fusarium Wilt in Muskmelon. J. G. LEACH and T. M. CURRENCE.

Muskmelon wilt, caused by a species of Fusarium and first observed in 1931, is slowly spreading through the melon-growing region near Minneapolis and St. Paul, Minnesota, and continues to be very destructive wherever it occurs. Numerous varieties of muskmelons have been tested for resistance on infested soil. The Honeydew and Persian melons were partially resistant, while other commonly grown varieties of muskmelon were very susceptible. One of about 30 plants selected in 1932 from a field of the Golden Osage type was apparently a chance hybrid between Golden Osage and Honeydew, and in 1933 yielded resistant plants of various types. Further selections were made and grown in 1934. Self-pollinated selections from these have been obtained for further study. The demonstration of resistance within the edible varieties of *Cucumis melo* lends encouragement to the selection of wilt-resistant types.

Uniform Rust Nurseries Indicate Decreasing Severity of Stem Rust. M. N. LEVINE and E. C. STAKMAN.

Observations on uniform rust nurseries, grown in various parts of the country and under diverse geographic and climatic conditions, show that there has been a gradual diminution in stem-rust losses during the past 15 years. While there was considerable variation from year to year, the severity of stem-rust infection was consistently decreased in both the wheat and oat nurseries, when comparisons were made by 5-year periods in the case of the former and by 4-year periods in the case of the latter. Reduction in severity of rust was further demonstrated by the reaction of individual varieties of wheat and oats in separate crop areas or in the entire nursery territory during the period under review. Several factors may be responsible for the reduction. During the past several years rainfall has averaged below normal. Nevertheless, some seasons were very favorable for rust development. Despite this, the losses were less than formerly. The eradication of about 20 million barberry bushes and the increasing use of resistant varieties of wheat and oats no doubt are very important factors in decreasing losses. (Cooperative investigations between the Minnesota Agricultural Experiment Station and the Division of Cereal Crops and Diseases, Bureau of Plant Industry, and the Division of Plant Disease Control, Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture.)

Cripple and Dark Stripe of Pineapples in Hawaii. M. B. LINFORD.

A disease, apparently identical with "cripple" in Queensland, is discussed as to symptoms, developmental sequence, and occurrence in Hawaii. Cripple is a fruit deformity resulting from partial or complete failure to develop one or more fruitlets. Dark stripe, on either surface of foliage or modified leaves, is an isolated, narrow, brown to black band of tissue devoid of epidermis, showing periderm activity. Absence of epidermis results both from tearing apart of adherent organs and from failure of the meristem to differentiate an epidermis. Dark stripe on floral bracts and sepals is closely associated with cripple, that on involucral bracts less closely, and that on foliage remotely

if at all. Dark stripe and mild deformity have been recognized in young inflorescences long before blossoming, the deformity increasing during growth. Neither mechanical injury nor pathogenic microorganisms seem involved. Cripple has appeared no more frequently in vegetative progeny of affected than of unaffected plants. Both cripple and dark stripe appear to arise from faulty differentiation at the apical primordium, but the basic cause is unknown. Cripple occurs widely in Hawaii, ranging from a trace to several per cent. No influence of culture or weather has been recognized.

Relation of Carbohydrate-nitrogen Nutrition to Disposition of Apple to Infection by Erwinia amylovora. G. K. K. LINK.

Stayman trees furnished by G. T. Nightingale and of identical past experience were grown in sand culture in Chicago tanks. Complete or plus-nitrate solution was applied once to one lot; to the other, instead, a minus-nitrate solution. The former lot developed succulent shoots with large dark leaves; the latter, shorter, thinner, but woody shoots with smaller, fewer, and lighter colored leaves. Both sets of shoots were inoculated simultaneously with *Erwinia amylovora* by spraying or by hypodermic injection. At inoculation the past experience of plants of the two sets differed only in application of 2.08 gms. of nitrate per plant. The former, the relatively low carbohydrate-high-nitrogen plants, were positively disposed, susceptible; the latter, the relatively high carbohydrate-low-nitrogen plants, negatively disposed, resistant: a demonstration of the rôle of past experience as a hygio- and pathogenic factor, respectively, in etiologic complexes of health and disease in plants. Susceptibility of the plus-nitrate plants was maintained through the season by their development of new shoots below lesions. Consequently, a negative correlative factor, the influence of a lesion in inducing development of a new susceptible region, was the only experimental variable in the etiologic complexes of the second and subsequent sets of lesions.

Suitability of the Chicago Soil-nutrient Temperature Tank for Phytopathologic Studies. G. K. K. LINK.

The Chicago botanical laboratory has developed the Wisconsin soil-temperature tank into a soil-nutrient-temperature tank. Glazed earthenware percolator urns permit constant drip or periodic application of solutions to soil or sand cultures. Coupled with use of air-temperature control, the tank readily permits simultaneous control of soil nutrients, water, and other hygiegenic and pathogenic soil ingredients; of soil pH and temperature; of air temperature; and coupled with use of shades and lamps, of carbohydrate nutrition. The containers avoid direct pathogenic effects of improper soil aeration, metal ions, and restricted root development and their indirect pathogenic effects in disposing plants to pathogenic influences. G. T. Nightingale, of the New Jersey Experiment Station, used the tanks at Chicago, demonstrating their suitability for analysis of the relation of ecic (environmental) factors and of the processes, structures, and behavior of apple trees. The author used them for study of the relation of carbohydrate-nitrogen nutrition of apples to disposition to infection by *Erwinia amylovora,* demonstrating their suitability for analysis of the etiologic complexes of infections of aerial parts of plants. Goldberg and the author, using sand cultures and varieties of cabbages resistant and susceptible to infection by *Fusarium conglutinans,* demonstrated suitability of the tanks for study of infection by soil-borne organisms.

Variability of Monosporic Cultures of Coccomyces hiemalis. ROBERT O. MAGIE.

The variability of 20 isolates of *Coccomyces hiemalis* from 6 States was studied. Length of conidia varied much within and between isolates. In culture the isolates

showed considerable variation in colony color and contour, growth rate, acid production, and spore production, both conidial and microconidial. No significant variation was found between the isolates in their utilization of various inorganic and organic sources of nitrogen and of several sources of carbon. Slight variation between isolates was demonstrated in the relation of temperature and hydrogen-ion concentration to growth and conidial germination. Conidia germinated over a range of pH from 2.9 to 8.5. Growth and production of conidia on agar occurred at temperatures from 4°–28° C.; none at 32°. The optimum temperature for growth lay between 20° and 24° C., and for conidial production at 12° or 16° C. Over a dozen isolates tested for pathogenic variability on several Prunus species were relatively uniform in their reactions, abundant infection occurring only on sweet and sour cherry. The isolates from any one locality varied as much as those from widely different localities. The existence of well-defined physiological forms of the fungus was not indicated.

Microorganisms in the Atmosphere of Arctic Regions. F. C. MEIER.

The Lindbergh Atlantic Survey Flight of 1933 presented an opportunity to obtain significant data concerning long-distance aerial movement of spores of fungi and pollen. Cooperating with the writer in studies on the relation of air-borne spores of fungi to the epidemiology of plant diseases, Colonel Charles A. Lindbergh, in the summer of 1933, made 26 collections of material present in the atmosphere at various altitudes over stretches of water and ice along his course of flight between Maine and Denmark. A device designed especially for exposing petrolatum-coated microscope slides from the low-winged monoplane *Tingmissartoq* while in flight, with slide containers so constructed as to prevent contamination before and after exposure, was used. Detailed notes and maps made at time of exposure provide a basis for interpretation of results as later revealed by direct examination of the slides under the microscope. The slides show certain spore types to be abundant in the air currents over Maine and Labrador and present in diminishing numbers as collections progressed to leeward over Davis Strait, the great ice cap of Greenland, and Denmark Strait.

Evidence of the Overwintering of Plasmopara australis in Fruits of Sicyos angulatus. I. E. MELHUS and G. C. KENT.

Plasmopara australis occurs annually on *Sicyos angulatus* growing on islands in the Mississippi River. Its abundance on the cotyledons of seedlings in late May and early June suggested the possibility of seed transmission. The mortality in the seedling stage is very high with abundant conidial sporulation. In the fall of 1933 the green fruit was found infected. Yellow areas and characteristic conidiophores were conspicuous symptoms. Material killed and fixed showed abundant mycelium in the ovary wall with conidiophores breaking through the outer and inner ovary wall. The conidiophores breaking through the inner ovary wall produced spores abundantly within the cavity between the ovary wall and the single mature seed. Spores were often found free in this cavity. There was no indication of oospores or of mycelium in the seed. The ovary wall encloses the seed until germination, which suggests a method of seedling infection.

A Practical Method of Organization for Long-time Phytopathological Field Experiments. I. E. MELHUS and C. S. REDDY.

An Albino Strain of Barley Smut. M. B. MOORE and C. C. ALLISON.

In the summer of 1934 a single head of barley infected with what appears to be an albino strain of *Ustilago hordei* was found growing in one of the field plots at University

Farm, St. Paul, Minnesota. The head, almost white, was intermediate between the loose-smut and the covered-smut types. The hyaline spores were smooth, somewhat smaller than those of normal *U. hordei*, and produced promycelia and sporidia on germination. Sporidial fusions between monosporidial lines indicate 2 sex groups within the albino strain that are compatible with those of normal *U. hordei* and *U. medians*.

The Distribution of Intermediate Types of Barley Smuts. M. B. MOORE and C. C. ALLISON.

To determine the distribution of barley smuts, about 700 samples of barley seed were obtained from Minnesota and about 125 from other States. Seed from these samples was sown in 1934 and the smutted heads, which appeared in about half of the plots, were examined for head type and color of spore mass. The spore-wall character and type of germination of the chlamydospores also were determined. Smutted heads collected in barley fields were similarly examined. Results show that, in addition to *Ustilago hordei* and *U. nuda*, *U. medians* is widely distributed from North Carolina to Colorado and from Missouri to Minnesota. More than one kind of smut often occurred in the same sample, occasionally two in the same head. Chlamydospores in one "loose smut" head were smooth and produced sporidia on germination. *U. nuda* and *U. medians* could not be differentiated by head type or color of spore mass. Heads determined as being smutted by *U. nuda* fell into at least two color groups.

Effect of Crown Rust on the Cold Resistance of Oats. H. C. MURPHY.

Winter-oat varieties and selections infected with crown rust were more susceptible to injury from artificially produced low temperatures than similarly hardened rust-free plants. Although the varieties and selections differed greatly in relative resistance to sub-freezing temperatures, it was always possible to select an exposure that was fatal to all infected plants of a particular variety or selection and that would allow a complete survival of rust-free ones. Plants with 10 per cent infection were easily differentiated. The heavier the infection, the greater was the susceptibility to injury. Tennessee numbers 090, 1918, 1962, 1922, and 1945 (C. I. Nos. 3175, 3172, 3174, 3171, and 3168, respectively) developed from Fulghum (winter type, C. I. 2499) and supplied by N. I. Hancock, were the most resistant of 30 selections studied, to low temperatures and were more resistant than any of 30 standard winter varieties. Hairy Culberson (C. I. 2505) and Culberson (C. I. 273), Coker 32–1 (C. I. 3026), Winter Turf selections (C. I. 435–4 and 409–5), Fulghum (winter types, C. I. Nos. 2499 and 2500), and Culred (C. I. 518–189) were the most resistant standard varieties. Some varieties hardened more quickly than others. Certain volunteer hybrid selections withstood $-16°$ F. under field conditions at Ames, Iowa, and survived after being transplanted to the greenhouse on January 24, 1934.

Effect of Crown Rust on Yield, Water Economy, and Composition of Oats. H. C. MURPHY.

Crown-rust infection of oats, initiated in the seedling and boot stages, entirely inhibited production of grain of the variety Markton (susceptible) and reduced the yield of Victoria (resistant) 47.5 and 30.8 per cent, respectively, while anthesis-stage infection reduced the yield of Markton 45.3 and of Victoria 18.3 per cent. Late dough-stage infection produced no significant effect. Root yield was reduced most and straw yield least. Susceptible plants infected in the seedling stage used 290.8 per cent more water per unit of dry weight than rust-free plants; resistant ones used 39.9 per cent more. Infection reduced the ratio of roots to tops. Effect on yield, water requirement,

and root-top ratio was in proportion to duration and severity of infection. Except for slight increase in crude protein and decrease in nitrogen-free extract for kernels, the composition of kernels and hulls was unchanged. Green, infected plants of Markton, Iogold (susceptible), Victoria, and Bond (nearly immune) showed an increase in percentage content of insoluble solids, soluble and insoluble ash and nitrogen, and acid-hydrolyzable substances, and a decrease in soluble solids, sucrose, glucose, levulose, and dextrin. The sugars were influenced most, levulose showing a decrease of 94.7 per cent in the infected Markton plants. The changes were in proportion to percentage and type of infection.

Three Forms of the Fusarium Wilt of Celery. RAY NELSON and L. C. COCHRAN.

Two forms of Fusarium wilt or yellows of celery occur generally in the celery districts of Michigan and also in a number of other States. An apparent third form, the distribution of which has not yet been determined, has recently been observed in California. These forms of Fusarium wilt have some characteristics in common, but are readily separated by the type and sequence of the initial symptoms in the leaflets. Of the two that are widely distributed the initial symptoms in the leaflets of form I are: (a) a progressive yellowing of the lamina, with the veins remaining unaffected much longer than the intervenal areas; (b) an absence of curl. In form 2 the initial symptoms include: (a) downward curling of the leaflets; (b) blanching of color from the veins and a narrow band of immediately adjacent tissue. The intervenal areas blanch much more slowly and the heart leaflets are seldom decolorized. In form 3 the leaflets usually are neither curled nor decolorized but the plants are dwarfed and exhibit other symptoms that show its relation to the other two forms of the disease.

Taxonomy of the Fusaria that Cause Celery Wilt (yellows). RAY NELSON and L. C. COCHRAN.

The pathogenic Fusaria associated with the wilt or yellows disease of celery fall into at least 2 groups. Two of these have been studied extensively and are morphologically indistinguishable from each other, nor are they clearly distinguishable on morphological grounds alone from other parasitic members of the subsection Orthocera. A third group is being studied, which apparently differs from the other two in certain morphological characters. The parasitic members of the subsection Orthocera differ morphologically from one another only in certain minor details. Apparently no great importance can be attached to the slight and variable differences in spore sizes, and specific separation on this basis appears unwarranted. The logical taxonomic procedure is to recognize only one species, *Fusarium lini,* and the other members as forms of this first named species. This treatment however, would contribute nothing to a better understanding of the relationships, nor would it provide a satisfactory classification for plant pathologists. Where clearly recognizable morphological differences are lacking, primary consideration should be given to host relationships in classification of the parasitic Fusaria. On this basis the 2 clearly recognizable groups of celery Fusaria are given, respectively, a specific and a varietal name, *Fusarium apii* and *Fusarium apii* var. *pallidum.* A third group requires further study.

A Study of Electric Soil Sterilization. A. G. NEWHALL.

Two general types of box sterilizers have been compared: the indirect, heating element, or New York type, and the direct, resistance, or Ohio type. Various kinds of soil, from pure quartz sand to muck, have been treated in over 100 test runs. Energy consumed was metered and reduced to kilowatt hours per cubic foot of soil per 50-degree

rise in temperature (21° to 71° C.), which amounted to less than 1.3 kw. hour. The optimum initial soil moisture, for most soils, was above 2/3 of its water-holding capacity d.w.b. The time required to raise the soil mass to 70° C. varied between 30 minutes and 6 hours, depending upon the sterilizer, soil type, its moisture and soluble salt content, and initial temperature. The following organisms have been placed in soils and killed, in varying lengths of time, by raising the soil temperature to 71° C. or less; *Heterodera marioni, Bacillus carotovorus, Rhizoctonia solani,* from several hosts, *Sclerotinia sclerotiorum, Phytophthora cactorum, Pythium ultimum,* and *Fusarium* sp. Ordinary soil-heating cable did not prove adequate for sterilization purposes, but pipe-type space heaters have been used in a limited way in bench soil.

A Study of the Carotenoid Pigments of Urediospores of Wheat Stem Rust and Four of its Color Variants. MARGARET NEWTON, H. JOHANNSON and T. JOHNSON.

Urediospores of stem rust and of four color variants of it (greyish brown, orange, white, and antique brown) were developed on wheat plants grown under normal and subnormal light intensities. Spectrophotometric determinations of the pigment concentrations of the naphtha extracts of spores of the above color types showed for each type a higher pigment content in spores produced under normal than under subnormal light. The concentration of pigment varied with the color type. Arranged in descending order of pigment concentration the types stood as follows: orange, normal, antique brown, white and greyish brown. Spectral distribution curves indicated that the pigments were carotenoid in nature, and in most instances apparently consisted chiefly of carotene.

A Cytological Study of the Resistance of Apple Varieties to Gymnosporangium juniperi virginianae. C. J. NUSBAUM.

Infections by *Gymnosporangium juniperi virginianae* in the leaves of 4 apple varieties, each differing from the other in its reaction to the rust fungus, were studied cytologically. The process of penetration by sporidial germ tubes was essentially the same on each variety. The fungus began its parasitic activities intracellularly by forming a conspicuous primary hypha in an epidermal cell. Further development of the mycelium was regularly intercellular, with the formation of characteristic haustoria. In Wealthy leaves of susceptible age, the parasite developed vigorously in congenial relationship with the host. As long as the invaded cells remained alive, they apparently furnished a favorable substrate. The resistance shown by the other varieties used seemed to be caused by a distinct antagonism of the host-cell protoplasts to the invading parasite. This reaction was mild in Yellow Transparent, pronounced in Fameuse, and very severe in Baldwin. Wealthy leaves became resistant as they matured. The fungus penetrated and made considerable vegetative growth in leaves that had just attained full size, but failed to initiate pycnia, in spite of an apparently congenial relation between the host and parasite. The sporidial germ tubes were unable to penetrate the epidermal cells of leaves several weeks older.

Dissociation of Fusarium niveum in Soil. C. R. ORTON.

During the months of March and August, 1933, 48 2-liter Ehrlenmeyer flasks were half filled with greenhouse soil of a sandy-loam texture. These were steam sterilized and 42 of them infested with 9 strains of *Fusarium niveum* from different sources. Six flasks were held as checks. The prepared flasks were distributed as follows: 16 in laboratory, 16 in greenhouse and 16 in a 6-inch soil trench outside. At approximately monthly intervals, samples were carefully removed from each flask and plated on agar.

Each colony thus obtained was studied in comparison with its respective "parental" strain. Three groupings were made: (1) colonies like parental strain; (2) colonies unlike parental strain, hereafter referred to as "colony dissociants"; and (3) colonies showing sectorial dissociation. Analysis has been made of 8,220 colonies isolated from the 48 soil flasks. Colony dissociants appeared 25 times and "sectorial dissociants" 16 times from 7 strains. Two strains have produced no dissociants. All of the remaining colonies were like the parental strains. Of the 25 colony dissociants 12 were distinctive—the other 13 representing replications. Of the 16 sectorial dissociants, 13 were distinctive. A number of the colony dissociants were apparently like sectorial dissociants obtained from the soil and/or stock cultures carried on plates in the laboratory.

Incubation of the Virus of Pea Mosaic in the Aphid, Macrosiphum gei. H. T. OSBORN.

The virus of pea mosaic, which has already been shown to require about 12 hours of incubation in the aphid *Macrosiphum pisi,* before it can be transmitted by this carrier, undergoes a similar period of incubation in the vector *M. gei.* An interval of not less than 12 nor more than 18 hours was found necessary for the proper incubation of the virus in this insect. The pea-mosaic virus is thus proved to enter into a specific relationship with two different aphids. A second virus affecting peas and other legumes, designated as pea-mosaic virus No. 2, is readily transmitted mechanically and is spread by both *M. pisi* and *M. gei.* This virus is transmissible by *M. pisi* within 30 minutes after being picked up, and is usually not retained for more than an hour. Its transmission by this vector seems to involve only a mechanical transfer.

Physiologic Studies of Several Pathogenic Bacteria that Induce Cell Stimulation in Plants. J. A. PINCKARD.

Atypical and pathological stimulation of plant cells has been investigated through certain bacteriological and physico-chemical studies of single-cell cultures of bacteria causing crown gall, olive tubercle, oleander tubercle, bacterial-pocket disease of sugar beet, and of an unnamed bacterium causing overgrowths on canes of black raspberry plants. The pathogenicity of the cultures varied on different host plants. The results of common bacteriological tests were usually differential. The optimum temperature for growth of all of the cultures on agar was near 28° C. They varied in amounts of growth with a number of sources of carbon and nitrogen. Alkaline reactions consistently occurred where starch and salts of organic acids were the sources of carbon. Marked differences in acid reaction developed where various other sources of carbon were present. Similar differences were noted in media containing several nitrogen sources. With oxamide, l-tyrosine and l-cystine an acid reaction was consistently induced by all of the cultures. Potentiometric oxidation-reduction studies in conjunction with H-ion determinations with glass electrodes showed that no limiting factors of pH were present in any of the media used and that strongly reduced potentials developed in undisturbed liquid cultures.

An Improved Method for Inducing Spore Fructification in Certain Species of Macrosporium. P. P. PIRONE.

In the course of studies on the leaf blight of carrots, caused by *Macrosporium carotae,* an improved method for inducing spore formation was developed. Air-drying of cultures for 24 to 48 hours at 21° C., with a relative humidity of 30 to 40 per cent, resulted in the production of large numbers of conidia. The age of the cultures used varied from 5 days to 4 weeks. They were grown on carrot, corn-meal, and potato-dextrose agars. The spores appeared in the air-dried dishes 48 hours after the Petri-dish covers were replaced. Undried cultures from the same source and of the same

age produced few to no spores. The present method produced a greater number of spores than did the wounding and shredding of young cultures with a hot scalpel as suggested by other workers. Danger of contamination by exposure was eliminated largely by use of a cellophane tent or covering placed a few inches above the exposed plates.

Bacterial Wilt of Corn and Its Insect Vectors. F. W. POOS and CHARLOTTE ELLIOTT.

During April, 1934, *Aplanobacter stewarti* was isolated from overwintered adults of the flea beetle, *Chaetocnema pulicaria.* Isolations have been made from about 40 species of insects, and *A. stewarti* has been isolated only from *C. pulicaria.* As many as 75 out of 100 *C. pulicaria* were found to contain *A. stewarti* when collections were taken from different host plants and localities under varying conditions in the field. Previously published reports of the transmission of *A. stewarti* from infected to healthy corn plants by means of the adult beetles of *C. pulicaria, C. denticulata,* and *Diabrotica duodecimpunctata* have been verified. Attempts to obtain infected plants in field cages by planting the seed in soil (both sterilized and not) inoculated with *A. stewarti* at planting time yielded only negative results. Bacterial-wilt lesions have been found on teosinte, *Euchlaena mexicana.* Bacteria isolated from these lesions have produced the disease on corn. Adult beetles of *C. pulicaria* also transmitted *A. stewarti* from infected corn plants to healthy teosinte, and pure cultures of *A. stewarti* were isolated from these infected teosinte plants.

Some Environmental Factors Influencing the Development of Apple Hairy-root and of Phytomonas rhizogenes. A. J. RIKER, D. H. PALMITER and E. M. HILDEBRAND.

The influence of temperature on the development of hairy root was studied during 4 spring seasons on 1-year-old Snow apple trees grown in Wisconsin temperature tanks. The trees were inoculated with *Phytomonas rhizogenes,* through wounds below the soil line. They were grown approximately 10 weeks in soil maintained at 75 per cent of its moisture-holding capacity and at 12°, 16°, 20°, 24°, 28°, and 32° C., respectively. The longest shoot growth appeared between 16° and 24° C. The largest hairy-root growth and the highest percentage of infection occurred at approximately 24° and 28° C. In the greenhouse kept near 24° C. inoculated trees were grown in soil at 60, 75, and 90 per cent of its moisture-holding capacity. Hairy-root development was slightly greater at 90 per cent, but the percentage of infection was slightly higher at 75 per cent. Colonies of the causal bacteria was grown on plates of yeast-infusion mannitol agar and of carrot-extract agar incubated, respectively, at temperatures between 4° and 36° C., inclusive. Widest colony growth was found at 28° C. In a yeast-infusion mannitol liquid medium the most acid and the most dense turbidity were produced also at 28° C.

Copper Phosphate Mixture: A Promising Fungicide. J. W. ROBERTS, L. PIERCE, M. A. SMITH, J. C. DUNEGAN, E. L. GREEN, and M. C. GOLDSWORTHY.

Field trials on apple, pear, and cherry during the 1933 and 1934 seasons at Beltsville, Md., Vincennes, Ind., Springfield, Mo., and Fayetteville, Ark., have indicated that the mixture composed of 2 pounds of copper phosphate (tri-cupric phosphate), 2 pounds of bentonite, and 4 pounds of hydrated lime in 50 gallons of water is a promising fungicide. The mixture has controlled mild to medium cases of apple scab, cherry leaf spot, and Fabraea leaf blight of pears, and has not caused injury to any of the leaves of these host plants. Slight russeting of fruit occurs with certain varieties. Slight stimulation was noted with all 3 hosts. The mixture has not been subjected to severe tests as a fungicide, and further trials are needed to establish its efficiency under the most favor-

able conditions of infection. Insecticides, spreaders, and stickers, of common use, may readily be incorporated in the mixture.

The Effect of Proteoclastic Enzymes on Purified Preparations of Tobacco Mosaic Virus. A. F. ROSS.

The virus was inactivated by trypsin. Heating the digests to 70° C. for 20 minutes resulted in a recovery of most of the virus. When enterokinase, calcium sulphate, calcium chloride, ammonium sulphate, magnesium sulphate, and barium chloride were used as activators for trypsin, permanent inactivation of the virus did not occur. It was impossible to adjust the H-ion concentration of the trypsin digests, within the limits that the virus remains active, so that permanent inactivation would occur. A combination of trypsin and pepsin was less effective in inactivation of the virus than trypsin alone. Papain inactivated the virus but it is not known whether or not this was due to adsorption or cleavage. Mixed cultures of bacteria caused a slow inactivation of the virus. Cultures of *B. proteus* and *B. aerogenes* were no more effective in inactivation than a mixed culture. Trypsin apparently attacked no protein that may be associated with the virus. Mixed cultures of microorganisms also were ineffective in digesting away any material associated with the virus.

Chemical Splitting of the Tomato "Combination-Streak" Virus Complex. MICHAEL SHAPOVALOV.

Expressed tomato or tobacco juice from plants infected with the "combination-streak" virus was mixed with equal amounts of different concentrations of a number of viricidal substances. Of the two components of this virus complex, green tobacco mosaic, known as Johnson's virus No. 1 (also sometimes referred to as virus A) showed, as a rule, higher resistance to these chemicals than latent potato virus (sometimes referred to as virus B). The usual method of separating this latter virus from the complex consists in employing such differential hosts as are subject to systemic infection with virus B alone. In a few chemical tests, however, certain sulphates, namely, $CoSO_4$, $NiSO_4$ and $ZnSO_4$, at concentrations incapable of producing lytic effect on the whole complex, appeared to be partically toxic to both component viruses. As a result, virus B occasionally survived, while virus A was inactivated, and *vice versa*. The limited number of trials thus far conducted show no regularity in this effect, and the outcome of these trials can not be predicted.

Influence of Nutrition on Host Susceptibility to Yellow Tobacco Mosaic. E. L. SPENCER.

Experiments have shown that susceptibility of seedlings of *Nicotiana tabacum* var. Turkish to yellow tobacco mosaic (Johnson's tobacco virus 6) can be influenced markedly by the addition of various amounts of nitrogen, phosphorus, and potassium. Plants were grown in 4-inch pots in a soil of low fertility and fed twice each week with their respective nutrient solutions. The pin-puncture method of inoculation was used. Susceptibility was measured by the number of lesions for each 100 pin punctures. Growth was measured by recording the green weights of representative plants at time of inoculation. Results showed that host susceptibility to virus infection in tobacco is definitely correlated with host nutrition. In the nitrogen study, plants that grew most rapidly were considerably less susceptible than those whose growth was retarded by excess nitrogen. In the phosphorus study, susceptibility increased when growth increased and decreased when growth fell off, due to excess phosphorus. In the potassium study, susceptibility was greatest in plants receiving small amounts of potassium but decreased sharply with

the addition of larger amounts; growth did not fall off appreciably until very large quantities of potassium were added.

Population Trends of Physiologic Forms of Puccinia graminis tritici, 1930 to 1934. E. C. STAKMAN, LEE HINES, ROBERT C. CASSELL, and M. N. LEVINE.

There have been striking changes in the relative prevalence of physiologic forms of *Puccinia graminis tritici,* based on determination of several hundred collections of rust in Mexico and the United States annually from 1930 to 1934, inclusive. In 1930 form 56 constituted only 0.2 per cent of all isolates, but its prevalence increased gradually until 1934, when it suddenly became the most prevalent of the 22 forms identified, constituting more than 30 per cent of the isolates. Similarly, the prevalence of form 34 increased from 0.7 per cent in 1930 to about 20 per cent in 1934. Form 139, first identified from an artificial cross, also seems to be increasing, but not so rapidly. On the other hand, the prevalence of form 38 decreased from an average of 34 per cent for the first 4 years to less than 4 per cent in 1934. There has been considerable fluctuation in the relative prevalence of other forms. It seems significant that all forms were identified from 29 aecial collections in 1934, while 21 forms were identified from about 700 uredial isolates. The ratios of forms to isolates therefore are about 1: 3 and 1: 33, respectively. (Cooperative investigations between the Minnesota Agricultural Experiment Station and the Division of Plant Disease Control, Bureau of Entomology and Plant Quarantine, and the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.)

Experiments on Physiologic Specialization and Nature of Variation in Ustilago zeae. E. C. STAKMAN, L. J. TYLER, G. E. HAFSTAD and E. G. SHARVELLE.

Six varieties of corn were inoculated hypodermically with 10 collections of *Ustilago zeae* three successive years. The collections differed consistently in pathogenicity. Because of the nature of resistance, however, physiologic specialization now appears relatively unimportant in breeding resistant lines of corn. Frequency of sectoring was studied in monosporidial lines derived from successively budded sporidia. In general, sectoring did not decrease appreciably, even in a line derived from the 13th successive sporidium. Apparently, sectoring was not due to delayed segregation. That tendency to sector is genotypically determined is shown by the following: On a promycelium segregation was of the 1, 2; 3, 4 type, culturally and sexually. In 89 colonies of 49 monosporidial lines from sporidia 3 and 4 there were 360 sectors; in about the same number of colonies of lines from sporidia 1 and 2 there were none. There were 8 types of segregation in 11 chlamydospores from a certain cross, including 3 that apparently remained diploid. Some monosporidial lines from the cross appear stable and are being combined to ascertain the importance of hybridity in sectorial variation.

Studies on the Soft-rot and Colon-typhoid-dysentery Groups of Bacteria. 2. Physiology. A. R. STANLEY.

A series of physiologic tests extending over a period of about two years were carried with 120 organisms of these groups. Cleavage of sugars, reduction of nitrates, production of indole, growth in Koser's citrate solution, methyl red test, and Voges-Proskauer test were tried. The indole test was the most consistent. Only one culture, after producing indole for some time, ceased to give a positive test in subsequent trials. It was found that 38 of these changed with respect to acid and gas production in one or more of the sugars. In later tests some of these reverted to their original reaction; while some have remained constant, a few have undergone further changes. Thus a

single organism could be classified at different times in two or even three different genera, according to Bergey's Manual. Our studies with these closely related bacteria indicate the inadequacy of the present classification (Bergey) as a basis for identification. Neither pathogenicity nor physiologic reactions can be fully relied upon for specific identification with such variable forms.

A Method of Determining the Rate of Decay in Wood. F. H. STEINMETZ and M. T. HILBORN.

The comparative loss in calorific value is suggested as a method of determining the rate of decay in wood. Since calorific value depends upon the chemical nature of the cell walls and cell contents, as well as upon the total amount of substances present, the method appears to be applicable. The actual combustion sample used was approximately 1 gram of pulverized oven dry wood or wood residue. Data are presented to illustrate the amount which fuel wood depreciated in calorific value over a 3-year period. The calorific values are based upon representative samples taken from pieces of split cord wood that had been stored in an open field. Three species of fuel wood were used in the experiment. The rate of loss is illustrated by the values obtained for red maple. By using $8.00 as the value of sound dry wood per cord cut in January and February the values for the 4 successive autumns were found to be $5.50, $3.40, $2.90, and $1.73 per cord. When expressed in percentages based upon millions of B. T. U. per cord, the efficiency of decayed wood was 70, 60, 59, and 38 for the 4 successive autumns following the time of cutting.

Further Evidence of the Seed-borne Nature of Peronospora destructor. W. W. STUART and A. G. NEWHALL.

Indirect evidence has accumulated indicating that various members of the Peronosporales are seed-borne. But, so far, only in the case of blue mold of tobacco and downy mildew of beet have infected seedlings been obtained from seed suspected of harboring the fungus. After repeated failures in the greenhouse to obtain diseased seedlings from onion seed suspected of harboring *Peronospora destructor* the authors were successful in one outdoor planting under conditions that were thought to point strongly to the seed-borne nature of this disease. Seed heads of Yellow Globe stock were inoculated early in the morning in the fall, while still in blossom, with a conidial suspension of *Peronospora destructor*. Natural infection also took place freely from a near-by onion field. These heads were hand-thrashed, fan-cleaned, and sown the following spring in 2 isolated virgin muck fields. On one of these plots, surrounded on 3 sides by dense woodland and 1200 feet distant from the nearest field of onions, mildew developed at about the same time that it was observed in other commercial plantings in the county. Fresh onion seed has been found to withstand treatment for 20 minutes in hot water at 55° to 57° C., but the effect on Peronospora is not yet known.

Laboratory and Field Studies on Sulphur as a Fungicide. J. J. TAUBENHAUS and PHARES DECKER.

A research program on uses for sulphur and sulphur combined with other products as fungicides for control of plant diseases has yielded some interesting results. Laboratory studies indicate that sulphurs of a 300 mesh are highly toxic to spore germination of pathogenic fungi. The toxicity is considerably increased by adding to the sulphur small percentages of other standard fungicides or insecticides. Sulphur may thus be made a valuable carrier to take the place of inert material as at present used in some fungicide or insecticide dusts. Sulphur applied broadcast to some of the calcareous po-

tato soils of Texas failed to give appreciable control of potato scab, while fair control was obtained with sulphur applied in the furrows with the seed pieces. Sulphur or sulphur in combination with Cuprocide or copper sulphate applied to sorghum seed heavily inoculated with smut spores of *Sphacelotheca sorghi* gave good control. Sulphur as a dust on the foliage of a number of plants controlled powdery mildew, and mosaic of the Lima bean and snap bean, presumably because of controlling red spider and leaf hoppers on the treated plants. Two to four per cent of sulphur worked into the ground, or sulphur slabs of pure sulphur, made perfect barriers to prevent the encroachment of Phymatotrichum root rot from an infested to a noninfested area. A large number of plants were dusted periodically with sulphur without injury to the foliage, while the leaves of most varieties of cantaloupes have been burned severely by sulphur dusts.

Further Studies on the Longevity of Sclerotia of Phymatotrichum omnivorum. J. J. TAUBENHAUS and W. N. EZEKIEL.

Tests were begun in 1929 to determine how long sclerotia of *Phymatotrichum omnivorum*, removed from the field and placed in small vials of moist soil in the laboratory, would remain viable and virulent. Each year some of the sclerotia have been removed from the vials and tested for germination. In 1934 18 per cent. of the sclerotia germinated after storage for 5 years at room temperature.

Particle Size of Three Strains of Tobacco-Mosaic Virus. H. H. THORNBERRY.

Particle size of tobacco-mosaic, aucuba-mosaic, and masked-tobacco-mosaic viruses was determined by ultrafiltration through Elford's type of collodion membranes. These viruses are considered to be related strains, although they cause diseases differing in severity of symptoms. In finding the end point of ultrafiltration (the smallest pores that allow virus to pass), the amount of virus in the filtrates was measured by the local-lesion method on Scotia beans. Under favorable conditions for filtration (0.1 M Sörensen's phosphate buffer at pH 8.5 and 10 per cent broth), membranes with pores .0905 μ in diameter allowed a small amount of each virus to pass. Membranes with pores .0329 μ in diameter retained the viruses. According to Elford's assumption, virus particles greater than ⅓ the pore diameter do not pass the membranes. On this basis, the virus particles of each strain were found to have a diameter not greater than .03 μ or 30 mμ.

Fasciation of Sweet Peas: A Bacterial Disease. P. E. TILFORD.

A bacterial organism, distinctly not *Bacterium tumefaciens*, has been isolated from numerous greenhouse-grown sweet-pea plants affected with the disease that has been called fasciation. Inoculation experiments have proved the pathogenicity of the organism to several varieties of sweet peas and to garden peas. The organism is seed-borne. Affected plants have many short, fleshy, thick, and aborted stems with misshapen leaves growing from the main stem at, or below, the soil line, or from the hypocotyl region. The main stem appears to grow in a normal fashion, except that it usually is somewhat dwarfed. No lesions of any kind have been noticed in connection with the disease on young plants. The identity of the organism is being worked out.

Severe Type of Mosaic on a Sugar-cane Variety. E. C. TIMS.

Of the 2 mosaic types occurring on a sugar-cane variety (C. P. 28–70) in Louisiana, one of them causes much more severe injury than is generally attributed to the mosaic disease. This type or strain of mosaic produces a very conspicuous, yellow mottling of the cane leaves, which are also greatly reduced in size. The infected stalks also are severely stunted. Yield tests made over a period of 2 years showed that tonnage was

reduced about *32 per cent* and the sucrose content of the juice was reduced *20–30 per cent* by this severe type of mosaic. The loss in tonnage is due to reduction in size rather than number of stalks. The yellow type of virus may be transmitted to other cane varieties on which the symptoms are similar to those of the ordinary green type of mosaic. The green or mild type of mosaic on the same variety (C. P. 28–70) apparently causes little or no reduction in growth and only a slight reduction in sucrose content.

Do Tobacco Plants Recover and Develop an Immunity from Ring Spot? W. D. VALLEAU.

The following hypothesis is suggested to explain so-called recovery and immunity from ring spot: Tobacco plants inoculated with the ring-spot virus develop ring patterns in inoculated leaves and other leaves until invasion of the growing point is complete. The Liesegang effect appears to result from the invasion of a mass of healthy tissue. Leaves developed subsequent to complete invasion are without patterns, but are parasitized and are highly viruliferous, as in any virus disease in which patterns are undeveloped. The upper leaves of a "recovered" ring-spot plant are darker green; growth is retarded; and, at low temperature, young leaves develop leaf-edge chlorosis and necrosis. Finally, abortive pollen is produced. Yellow ring spot is identical with ordinary ring spot, except that patternless leaves bleach to a yellowish green, proving that recovery has not taken place. Inoculation of patternless leaves is without effect, as the cells are already parasitized to the limit. An acquired immunity is not involved. For the same reason, tobacco plants inoculated with a mild strain of tobacco mosaic are protected against a more injurious strain of the same virus.

The Resistance of Ambalema Tobacco to Different Viruses. W. D. VALLEAU.

Nicotiana tabacum var. Ambalema, found to be highly resistant to ordinary tobacco mosaic by Nolla, is susceptible to cucumber mosaic and green and yellow ring spot and slightly resistant to the etch viruses (smallest growing-point leaves free from virus). Ambalema plants were inoculated with 27 strains of tobacco mosaic, each differing in symptom expression. The majority caused only faint vein clearing, occasional faint patterns and yellow blotches, but the plants, on the whole, appeared healthy. Four strains caused chlorotic (in one virus necrotic) Liesegang patterns, often covering the leaf. The ring-spot patterns evidently resulted from slow penetration of healthy leaves, as in nearly every case the growing-point leaves were virus-free. Ambalema appears to separate strains from a given supposedly stable tobacco-mosaic virus, the process appearing not unlike that involved in the production of so-called attenuated strains by heat. An Ambalema plant may be shown to have been inoculated successfully several consecutive times if young leaves are used, and a strain of tobacco mosaic employed that causes local chlorotic patterns. A strain of tobacco mosaic (white) collected in Kentucky and the English aucuba mosaic each produce necrotic spots and concentric necrotic ring patterns on inoculated leaves, the virus appearing to be localized.

Technique Advantageous for the Isolation of Ceratostomella ulmi from Bark Beetles. J. M. WALTER.

Attempts to isolate the Graphium stage of *Ceratostomella ulmi*, the Dutch elm-disease fungus, from bark beetles by the agar-plate method have been highly unsuccessful because the agar is quickly overrun by fast-growing fungi, such as species of Mucor, Aspergillus, Monilia, and Fusarium. These apparently occur in the alimentary tracts, because washing the beetles for 5 minutes in 1: 1000 $HgCl_2$ did not eliminate them; nor did such modifications of common media as adjusting pH, reducing sugar content, and changing nitrogen base. Recently it has been found that the coremia of *C. ulmi* develop well and

that other fungi are checked if the beetles are crushed on sterilized chips cut from seasoned, debarked, elm branches about ⅝ inch in diameter. Wood seasoned for 2 or 3 months is less favorable for growth of the unwanted fungi than is freshly cut wood. The chips should preferably be autoclaved dry on 2 or 3 sheets of filter paper in Petri dishes. Sufficient sterile water to moisten the chips and filter paper thoroughly is added a few minutes before the plates are used. This method also has proved advantageous in the isolation of *C. ulmi* from dead wood and bark. Several other species of Graphium, not easily isolated by the agar-plate method, have been also secured. (Emergency Conservation Work in cooperation with U. S. Department of Agriculture.)

An Aerial Rhizoctonia on Beans. G. F. WEBER.

A bean disease, new in the United States, was observed in epidemic form during the past 2 years in Alachua County, Florida. The disease occurs on all aboveground parts of the host plants, but is most destructive on the foliage. Leaf blades are killed from the point of attachment of petiole and blade as the fungus advances over them in typical thread-blight fashion from the stem and petiole or from an invaded part to a noninvaded part when there is contact. The hyphae apparently are both subcuticular and superficial. The former invades the host cells, killing them; the latter spreads over the leaf-blade surfaces. The killed areas first appear scalded, then dry out, often becoming brown. Hosts found infected are the Bountiful variety of string beans, Fordhook Lima beans, cockle bur, *Xanthium americanum,* and groundnut, *Glycine apios.* This disease is very similar to that described on fig and caused by *Rhizoctonia microsclerotia.* It and a blight disease of Japanese varnish tree, *Firmiana simplex,* are probably identical. The sclerotia produced in nature on bean and fig are quite comparable. The sclerotia produced in cultures by the fungus from the bean are larger and more irregular than those produced on the host.

A Fungus Spot of Azalea Flowers. FREEMAN WEISS.

In 1931, flowers of cultivated azaleas, *Azalea indica,* near Charleston, S. C., were devastated by a hitherto unknown disease. Within a few days of its first appearance flowers by thousands became spotted, then collapsed. The disease has since recurred annually, always coincident with the period of maximum bloom, though varying from late February to mid-April. Only flowers are affected; from adjacent buds healthy leaves emerge and the bushes exhibit no general malaise. Among various organisms isolated from affected flowers (Alternaria, Botrytis, Cladosporium, Pestalotia, yeasts, bacteria) a fungus of exceptional morphology was found which proved upon experimental inoculation solely responsible for the characteristic features of the disease. Within the flower tissue this fungus produces a coarse, septate mycelium in whose train the cells are thoroughly disorganized. Conidia suggestive of Ovularia are first produced, then microconidia typical of Sclerotinia. Subsequently cupulate, smooth, black sclerotia are formed. Microconidia and sclerotia, but not conidia, form readily also on artificial media. Indications of heterothallism and of immature iscomycetous fruit bodies in cultures have been observed. The anticipated rôle of the flower sclerotia has not proved susceptible of experimental proof, outbreaks of disease occurring where they are only a distant factor. The agency of bees (Apis, Bombus, Xylocopa) in disseminating and inoculating the fungus into flowers has been demonstrated.

Bottom Rot of Calla Rhizomes. FREEMAN WEISS and THELMA B. POST.

The rhizome of *Zantedeschia aethiopica* is an upright fleshy stem of indeterminate apical growth; its basal portion dies back as the crown expands and ceases to produce

roots or shoot buds. The moribund basal portion typically is not delimited from the vital middle and crown region by an abscission layer, a condition that invites fungous invasion. In the commercial handling of callas in Pacific Coast nurseries, especially in their transportation to eastern markets, extensive spoilage from bottom rot—a combination of physiological and pathological factors—has occurred in recent years. The Godfrey variety is especially subject to this deterioration. Among the commonly associated fungi are Alternaria, *Phyllosticta richardiae, Fusarium* spp. (Sect. *Martiella*), *Rhizoctonia solani* and a Phoma (in von Höhnel's sense) not hitherto recorded on this host. Bacterial soft rot has little, if any, part in the complex. *Phytophthora richardiae,* which causes an aggressive rhizome rot when inoculated into wounds, cannot ordinarily be detected in nor isolated from natural cases of bottom rot. The Phoma is definitely capable of wound parasitism. Alternaria and Phyllosticta appear to be only casually associated with infected rhizomes, but both are parasitic on aerial organs. Rhizoctonia and the *Fusarium* spp. studied are not pathogenic to growing plants; their rôle in bottom rot of dormant rhizomes is still undetermined.

Serically Active (Haptenic) Carbohydrates of Genotypes of Neurospora tetrasperma and N. sitophila. HAZEL W. WILCOX and GEORGE K. K. LINK.

Polysaccharides isolated from two genotypically self-sterile, inter-fertile strains of *Neurospora tetrasperma* (obtained from B. O. Dodge) reacted positively in precipitin-ring tests with antiserums engendered in rabbits following injection with saline extracts of powders of these strains. Specific soluble carbohydrates isolated from each of the 8 monoascosporous strains of a single ascus of *N. sitophila* (obtained from Dr. Dodge) reacted positively in precipitin-ring tests with each of the 8 antiserums of these strains. Work is under way to determine whether all or some of these strains of *N. tetrasperma* and of *N. sitophila,* respectively, in addition to their common carbohydrates, possess strain-specific carbohydrates. (This research was aided in part by a grant to the University of Chicago by the Rockefeller Foundation.)

Host-Parasite Relationship in Bean Rust. S. A. WINGARD.

Histological studies of rust-infected bean leaves indicate that bean varieties commonly regarded as being rust resistant are hypersensitive. The leaf tissues of the hypersensitive hosts die when invaded by the rust fungus and thereby automatically destroy the fungus before any spores can be produced. In the case of the susceptible bean varieties, the invaded tissues of the host are not destroyed by the rust fungus; but, on the contrary, are stimulated and preserved at the expense of the uninvaded tissues. The colonies of invaded host cells, together with the invading rust hyphae, form the parasitic units which live at the expense of the surrounding host cells. This symbiotic relationship between rust hyphae and invaded host cells continues until after the sori and spores have been produced and the surrounding tissues are too much impoverished to continue to nourish the parasitic units. The rust hyphae in the last stages of spore production lose their ability to take the stain, as if completely exhausted, whereas the invaded host cells seem to be invigorated, as shown by the production of a green island surrounding the sorus.

Sclerotinia Rot of Pumpkin and Squash. P. A. YOUNG.

Sclerotinia caused fruit rot in 4 varieties of squash and 3 varieties of pumpkin in Bozeman, Montana, last year. The rot appeared in the field and in storage. Most of the rotting spots did not touch the soil. In squash, Sclerotinia caused disc-shape, rapidly enlarging, water-soaked spots, 2 to 10 cm. wide. Exuded droplets of yellow sap

studded the margins of many lesions. White mycelium protruded from the centers of some of them. The squashes became dry mummies, containing many large sclerotia. Sclerotinia caused a rapidly developing soft rot of pumpkins, releasing large amounts of liquid and producing dense masses of white mycelium with many black sclerotia that ranged from 0.5 cm. to 15.5 by 12 by 0.5 cm. in diameter. Sclerotinia decomposed most of the seeds in these rotted curcurbit fruits. Sap, exuding from mechanical wounds, may serve as a culture medium for Sclerotinia ascospores, thus facilitating wound infection. A Sclerotinia was isolated from the naturally infected curcurbit fruits, and produced typical rots when inoculated into wounds in healthy pumpkin and squash fruits; it was reisolated from them. Sclerotia from pumpkins produced typical apothecia and spores of *Sclerotinia sclerotiorum.*

A New Variety of Tilletia tritici in Montana. P. A. YOUNG.

Nontreated Turkey wheat, planted in a 200-acre field in 1931, produced wheat with 49 (range 25 to 95) per cent of the heads bunted. Study during 3 years showed that this bunt is a new variety of *Tilletia tritici.* Soil infestation with it caused 0.2 to 4.9 per cent of bunted heads in 3 varieties of winter wheat in an experimental plot. The characters given below distinguish this new variety from *T. levis* and from the other forms of *T. tritici* that occur in Montana. The chlamydospores of this variety are 13 to 18 microns in diameter, usually have very prominent reticulations, and rarely germinate in water at 12° or 25° C. Most of the bunt balls were hard and solid instead of soft and powdery inside. Bunted plants commonly had more tillers than normal plants. Nearly all of the bunted heads were borne by extremely dwarfed stems only 3 to 18 cm. tall. They were short and resembled normal heads with their wide-spreading awns. Seed inoculation caused no bunt in 6 varieties of spring wheat and 9 varieties of winter wheat, but they caused nearly 1 per cent of bunted heads in 5 other varieties of winter wheat.

Insoluble Copper Compounds as Substitutes for Bordeaux. H. C. YOUNG and J. R. BECKENBACH.

Two years' laboratory and field tests indicate that basic copper sulphate, copper oxi-chloride, copper phosphate, and Coposil have limited value as fungicides. Their major limitation—lack of adhesiveness—can be largely corrected by the use of Bentonite and a specially treated clay called Wyojel. The ordinary clays and stickers, such as Kaolin, Bancroft and Dawson clay, Talc, flour, and Fluxit, increase the adhesiveness of the insoluble coppers about one-half of that of the Bentonite group. The results from field tests on cucurbits indicate excellent promise for these copper compounds. The most effective formula used so far was a 1–5–1; that is, 1 pound of the copper compound, 5 pounds of fluffer or filler, and 1 pound of calcium or lead arsenate. The results from preliminary tests on potatoes and celery indicate that the addition of lime decreased the fungicidal value. All of these insoluble copper compounds caused serious injury and russeting of apples in northern Ohio.

THE LIFE HISTORY OF TAPHRINA DEFORMANS

A. J. Mix[1]

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REVIEW OF LITERATURE

Before presenting the results of recent experiments and observations on the life history of *Taphrina deformans* (Fkl.) Tul.,[2] it will be necessary to give an account of previous investigations. This will involve some discussion of other species, especially as regards occurrence of perennial mycelium.

References to this fungus in the earlier mycological literature are chiefly concerned with its occurrence, morphology, and taxonomic position. If the early mycologists were interested in the life history of species of Taphrina they perhaps believed that new infections were traceable to ascospores. De Bary (3) states that in the case of *Taphrina pruni* (Fkl.) Tul., infection is probably accomplished by "*sprossen*" derived from ascospores, but that the manner of infection is not known. Ráthay (20), after a careful study of the distribution of mycelium of *Taphrina pruni* in stem and leaf, decides that this mycelium is not perennial and that new infections are due to spores. The essential points of Ráthay's observations are: (1) Mycelium could never be found in parts of twigs older than the current season's growth; (2) foliage shoots were observed with only the tip diseased or with more than one diseased portion separated by sound portions; (3) diseased shoots were found arising from axillary buds on twigs that were entirely sound and free from mycelium; (4) mycelium could never be traced from a diseased twig out into the new foliage shoot arising from it, and such new shoots might be either healthy or diseased; (5) it was observed that infected parts of stems invariably died, coincidently with the death of infected leaves.

Ráthay (21), in a subsequent paper, reports perennial mycelium for *Taphrina cerasi* (Fkl.) Sadeb., (*Exoascus wiesneri* Ráthay). He found, in winter, mycelium present in primary and secondary bark, pith, rays, and cambium of every part of the witches' broom from the youngest shoots to those three or more years old. In summer he traced hyphae from the

[1] The writer wishes to acknowledge a grant from the Research Committee of the Graduate School, University of Kansas, by means of which part of the cost of publishing the illustrations accompanying this paper was defrayed.

[2] In previous publications the writer has referred this fungus to *Exoascus deformans* Fkl., following the classification of the Exoascaceae proposed by Sadebeck (27). The characters used by Sadebeck to separate genera seem to be perfectly reliable. Nevertheless, most mycologists do not follow his classification and recognize but one genus, Taphrina.

hymenium on the leaves to the mycelium within the stem. This is the first circumstantial account of perennial mycelium in the Taphrinaceae and it should be emphasized that in this case the host deformation is a perennial structure. Ráthay's findings were confirmed by Kutsomitopulos (13).

Sadebeck (24), as the result of observations on *Taphrina tosquinetii* (West.) Magn. (*Exoascus alnitorquus* (Tul.) Sadeb.) and *T. ulmi* (Fkl.) Sadeb. (*E. ulmi* Fkl.), states that in these forms the mycelium is perennial beneath the cuticle in the buds. In the taxonomic portion of his paper he includes *T. deformans, T. pruni,* and two other species in a group in which the mycelium is perennial in the inner tissues of the twigs. These statements of Sadebeck rest on careful observations in the case of *T. tosquinetii,* but for the other forms mentioned they are pure assumptions.

Johanson (10), after careful study of the distribution of mycelium in the host, decides that *Taphrina tosquinetii, T. betulina* Rostrup, and *T. borealis* Johans. possess perennial mycelium, while *T. carnea* Johans., *T. sadebeckii* Johans., and probably also *T. betulae* Fkl., do not. It is worth considering that these last named species affect only the leaves and stems of the current season's growth and do not form witches' brooms or other malformations on the older parts of the host.

Johanson also suggests that in species of Taphrina, whether possessing perennial mycelium or not, the bud conidia derived from ascospores may overwinter and cause infection the following spring.

Sadebeck (25) disagrees with Johanson as to the lack of perennial mycelium in the species mentioned above but presents no evidence. In a later paper Sadebeck (26) states that in species possessing only subcuticular mycelium this mycelium overwinters in the buds, while in species whose mycelium penetrates deeper into the host it is perennial in the older tissues of the shoots. He repeats his account of observations on *Taphrina tosquinetii,* retracts his former statement that *T. ulmi* has perennial mycelium, and again, in his taxonomic account, divides the forms with perennial mycelium into those in which it overwinters in the buds, and those in which it overwinters in the stems. He places *T. deformans* in the latter group, stating that the mycelium overwinters in the youngest parts of the one-year twigs, and may be found in the primary bark, pith, and rays of the first shoots of each vegetative period, growing out into the leaves and ultimately forming the subcuticular hymenium. He does not report having found mycelium in new stems earlier than in leaves, and it is important to realize that his account of the distribution of mycelium in leaves and stems may be interpreted as indicating that this mycelium has entered the stem from the leaf. In fact, Sadebeck's contributions concerning perennial mycelium in the Taphrinaceae adduce evidence of its occurrence in one species, *T. tosquinetii,* and present ungrounded assumptions of its occurrence in several other species, including *T. deformans.*

Atkinson (2), apparently following Sadebeck, asserts the presence of perennial mycelium in *Taphrina deformans, T. cerasi, T. pruni, T. mirabilis* Atk. and *T. decipiens* Atk. In his general discussion of pruniculous Taphrinaceae Atkinson states . . . "the species possess a perennial mycelium." He says further that the mycelium of *T. deformans* passes the late summer, autumn, and winter in the tissue of the leaf buds, growing out in the spring into the developing leaves, and believes that this explains the fact that buds taken from affected trees transmit the disease to nursery trees. These statements of Atkinson rest neither on cited authority (except in the case of *T. cerasi*) nor on recorded observations.

Duggar (6) points out that, occasionally, twigs affected by peach leaf curl do not die but make new healthy growth above the swollen diseased portion. He shows a photograph of twigs that have thus outgrown the disease and states that the fungus overwinters in the swollen diseased parts of such twigs. He does not report having found this overwintering mycelium. Duggar suggests two other possible ways in which the fungus may survive: (1) By spores infecting the buds immediately after dispersal, (2) by spores overwintering on the twigs, in the bud scales, or on the ground, and infecting the opening buds in the spring. His spraying experiments support the latter idea, protection being secured when trees were sprayed before the buds began to swell but none from spraying after the leaves emerged.

The spraying experiments of Pierce (19) show that infection by overwintering spores must occur, and Pierce believes that these are responsible for nearly if not all curl. Since a single spraying gave 97 to 98 per cent control, only 2 or 3 per cent of the curl of a given season could possibly be due to overwintering mycelium. Pierce points out that Sadebeck gives no account of experimentation to support his assertion that the mycelium of *Taphrina deformans* is perennial. Pierce's own studies of the distribution of mycelium in diseased twigs lead him to believe that mycelium enters the twig from the leaf, especially as it diminishes in amount with distance from the diseased petiole.[3]

Recently Fitzpatrick (8), by means of spraying experiments has determined closely the time of infection in two seasons in eastern Ontario, and has correlated these infections with rain periods. He concludes that leaves are susceptible when emerging from the bud and for a short time thereafter, a fact that has been fully recognized since the spraying experiments of Duggar and Pierce.

Following Pierce's work most pathologists have accepted the idea that infection is due to overwintering spores lodged on the buds or twigs and

[3] Pierce cites a number of earlier authors, such as Frank and Sorauer, who assume that mycelium of *Taphrina deformans* is perennial. These citations are omitted from the present account, since they offer no experimental proof.

that perennial mycelium, if present, can be responsible for only a small proportion of curl. However, the exact manner of infection by spores has been in doubt.

The writer's earlier work (15) proved that conidia derived from ascospores by budding may infect nascent leaves and even those that are out of the bud and considerably enlarged. The natural occurrence of late infection on large leaves is reported in a subsequent paper (16).

The writer has further shown (15) that conidia are capable of withstanding desiccation for periods long enough to explain their survival from the time of ascospore dispersion until that of infection. That survival in nature is aided by a saprophytic multiplication of conidia is a reasonable assumption. The fungus thrives in culture in a wide variety of media, many of them of low food content, as, for example, a very dilute solution of peach gum, and it is difficult to believe that it may not find occasional opportunity for saprophytic growth in solutions naturally occurring on peach twigs. There should, in fact, be no more mystery about the survival from one season to the next of the saprophytic bud conidia of *Taphrina deformans* than there is about that of yeast cells.

ATTEMPTED ISOLATIONS FROM TWIG SURFACES

It would be gratifying if direct evidence of the presence of overwintering conidia on peach twigs could be obtained by means of isolations from twig surfaces. A number of unsuccessful isolations have been previously reported (15). An attempt has since been made to recover the fungus from twig surfaces when it was known to be there. On Nov. 2 and 29, 1926, several branches of peach trees were sprayed with spore suspensions of *Taphrina deformans* and plate isolations were made on Nov. 30, Jan. 6, and Feb. 24. Numerous colonies of both budding and filamentous fungi developed in these plates and many transfers were made from the budding colonies. In this manner 4 cultures of *T. deformans* were obtained, all from isolations made on Nov. 30. None was secured from the later isolations. That the fungus did, however, survive on these twigs is indicated by counts made on April 28, which showed, on the inoculated branches, 165 curled shoots and 132 healthy, while on branches subject only to natural infection (selected at random but avoiding those close to the inoculated branches) 20 curled and 580 healthy shoots were found.

The difficulty attending these attempted isolations is, as already pointed out (15), due to the occurrence on twig surfaces of budding and filamentous fungi all of which grow faster in culture than does *Taphrina deformans*. It does not seem possible to obtain a differential medium for this fungus. On any medium that has been found to support its growth, saprophytic forms grow as well or better.

Since the curl fungus grows (15) in highly acid media, isolations from twig surfaces were attempted on acidified potato-dextrose agar (pH 3.5). This also allowed growth of many saprophytic forms and the curl fungus was not obtained.

The procedure in these isolations was varied by distributing a few cubic centimeters of twig washings in a large volume of medium (500 to 1000 cc.) and using all the medium in pouring plates. This resulted in less crowded plates but did not allow isolations of the curl fungus. Fitzpatrick (8) seems to have succeeded where the writer has consistently failed.

INOCULATION EXPERIMENTS

Successful inoculation experiments in which cells from pure cultures of *Taphrina deformans* were used have previously been reported (15). Since no facilities were at hand for keeping potted trees in the greenhouse under conditions sufficiently cool and humid to insure infection, these inoculations and those to be reported below were performed on trees growing out of doors. Young Elberta trees were obtained from a nursery in the spring of 1922 and planted on the university campus. The nearest peach tree was over a quarter of a mile distant and there were no peach orchards within a mile. Certain branches on these trees were sprayed with Formalin 1–200 and subsequently inoculated in the spring of 1922 and again in 1923 (15). The curled leaves were carefully removed and destroyed each season before ascospores were formed. The trees were not inoculated in 1924 or 1925 and there was no curl on them in those years. It was, therefore, thought safe to omit the spraying with Formalin in the following inoculation experiments. A few branches on these trees were inoculated in October, 1925, and in January, March, and April, 1926, but no curl resulted. These were very unfavorable seasons for curl, as will be explained more fully below.

In 1926–27 a large number of branches were inoculated, in July, November, January, February, March, and April. Two methods were used, (1) smearing the buds with the yeast-like mass of cells obtained in culture, (2) spraying twigs and buds with a suspension of cells in sterilized water. Two cultures of *Taphrina deformans* were used, one (No. 11), isolated in 1920 and the other (No. 101), isolated in 1926. For smear inoculations these cultures were used alternately; in the spray inoculations they were mixed.

In making the counts no attention was paid to severity of infection, a shoot being counted as "curled" whether there was one curled leaf or many. In most cases, however, several leaves of each shoot showed curl.

The results (Table 1) indicate that conidia from culture may survive on the twigs from mid-July (approximately one month after ascospore dispersion) to the following spring, and still cause infection. While most

TABLE 1.—*Results of inoculations of peach branches with Taphrina deformans, 1926–27*

Branch	Date	Method	Culture	Curled shoots	Healthy shoots
97	July 13, 1926	Spray	11 and 101	29	8
2	Nov. 2, "	"	" " "	27	2
77	" " "	"	" " "	83	19
23	" " "	Smear	11	58	11
94	" " "	Spray	11 and 101	117	23
40	" " "	Smear	101	46	0
34	" 29 "	Spray	11 and 101	135	62
47	" " "	"	" " "	46	27
58	" 30 "	Smear	11	13	3
59	" " "	"	" " "	17	1
38	" " "	"	" " "	13	3
18	" " "	"	" " "	16	2
26	" " "	"	101	24	3
3	" " "	"	"	18	0
6	" " "	"	"	10	0
19	Jan. 5, 1927	Spray	11 and 101	25	30
32	" " "	"	" " "	10	17
17	" " "	Smear	101	10	25
83	" " "	"	11	1	10
7	" " "	"	"	12	8
80	" " "	"	"	19	8
46	Feb. 22, "	Spray	11 and 101	100	35
4	" " "	Smear	11	22	39
85	" " "	"	"	29	10
62	" " "	"	101	22	15
56	Mar. 9, "	"	11	63	35
79	" " "	"	101	25	6
8	" 15, "	"	"	3	24
29	" " "	"	11	7	40
13	" 19, "	Spray	11 and 101	175	125
5	" 31, "	"	"	36	157
		Total		1211	748
		Percentage curl		62	
		Control branches near inoculated branches		84	260
		Percentage curl		21	
		Control branches remote from inoculated branches		95	730
		Percentage curl		11	

of the inoculations of this season were made on branches not inoculated the previous year, branch number 40 was inoculated on Feb. 23, 1926, and again on Nov. 2, 1926. It will be noticed (Table 1) that every shoot on this branch became infected in 1927. Facts not shown in the table are

that every leaf of every shoot was curled, and many shoots showed distortion of the twigs by the curl fungus. The unusual severity of the curl on this branch may well be explained by the larger amount of inoculum, and may also be taken to indicate that conidia from culture survived on the twigs from February, 1926, until the spring of 1927.

The occurrence of curl on the control (noninoculated) branches of these trees may be taken as further evidence of the survival of conidia for more than one year. The remoteness of these trees from those affected by curl, and the near absence of natural curl in this locality in 1926, suggest that the curl on these noninoculated branches in 1927 was due to inoculations in the preceding year. Even if it is considered to be due to natural inoculation, survival of conidia for more than one year is, nevertheless indicated, since there was no curl whatever on these trees in 1926.

In the spring of 1929 peach seedlings bearing dormant Elberta buds were obtained and planted in pots in the greenhouse. The seedling tops were cut off and the stumps sprayed with Formalin 1–200. After the new Elberta shoots had made considerable growth the trees were again sprayed with Formalin and, on July 9, a few days later, were inoculated by the spray method. These trees were kept in the greenhouse until early September, when they were planted outside. Counts of curled shoots (Table 2),

TABLE 2.—*Results obtained from potted trees inoculated with Taphrina deformans. The inoculations were made on July 9, 1929, and the results were recorded on May 15, 1930*

Tree No.	Culture	Curled shoots	Healthy shoots
1	4	13	17
2	C1	12	16
3	C7	14	16
4	1	32	44
5	2	28	16
6	3	30	24
7	Control	0	42

made in May, 1930, indicate that conidia had survived on these trees from July until the following spring. Wieben (30) reports inoculating peach buds with pure cultures of *Taphrina deformans* in the summer, with infection resulting the following spring.

Bagging Experiment

On April 30, 1927, a number of peach shoots were enclosed in transparent, waterproof paper bags. The shoots chosen were free from curl.

Nearby shoots bore many curled leaves approaching the stage of ascospore dispersion. Ejection of ascospores began about May 2 and was nearly finished by May 20. The bags were left on until June 11, when all curled leaves had fallen. Bags on branches 13 and 19 were left in place during the summer and were finally torn open by wind and rain in the fall. The results of the bagging experiment (Table 3) indicate that when spores from

TABLE 3.—*Results of bagging peach shoots on trees infected with curl*

Shoot No.	Date of bagging	Bag removed	(1928) Curled shoots	(1928) Healthy shoots
23	April 30, 1927	June 11, 1927	0	6
2	" " "	" " "	1	5
21	" " "	" " "	0	5
6	" " "	" " "	0	12
18	" " "	" " "	Shoot dead	—
9	" " "	" " "	0	13
19	" " "	Not removed	0	9
13	" " "	" "	Shoot dead	—
Controls—Shoots chosen at random in these trees in the spring of 1928			55	630

curled leaves are prevented from reaching peach twigs, curl does not result the following spring.

SUMMER SPRAYING

Reddick and Toan (22) have shown that fall spraying effectively prevents curl. It seemed possible that summer spraying also might prove effective, and furnish additional evidence of the spores on twig surfaces. Facilities were not at hand for spraying on a field scale but a few branches of affected trees were sprayed with lime-sulphur 1–100 on May 19, 1927 (during the period of ascospore dispersion), and others on June 25 and June 27 (after ascospore dispersion had ceased). Counts made in the spring of 1928 showed 21 curled shoots to 290 healthy (6.8 per cent curl), but counts of shoots chosen at random on unsprayed branches showed 23 curled shoots to 315 healthy (6.9 per cent curl). The experiment was quite inconclusive, and, because of the difficulty in spraying the twigs thoroughly when there is an abundance of foliage, no further experiments were attempted.

FIELD OBSERVATIONS

The development of peach leaf curl in eastern Kansas in 1924 has been previously described (16). Infection occurred only after the leaves were partly grown and the total amount of curl was much smaller than usual.

In 1925, although there was a prolonged rain when the peach buds were pushing and two later rain periods occurred, there was not so much wet spring weather as is usual in this locality. Curl was more abundant than in 1924 but much less abundant than in most seasons.

In 1926 there was no prolonged rain during the period of susceptibility. Inoculation experiments made this spring failed (as noted above) and only a very few curled leaves could be found in orchards usually badly infested.

The occurrence of curl in 1927 has been briefly described elsewhere (16). Following two years of scarcity of curl, and a third year in which it was almost entirely absent, the outbreak in 1927 was the most severe during the ten-year period of the writer's observation. Rain periods of six or more hours' duration occurred on March 10 (peach buds showing green tips), March 19, (leaves ¼ inch out of buds), March 19–20, March 30–31, April 6–7, 8, 12, and 13–14. Curl in its early stages was very prevalent by April 15. On April 27 orchards in this vicinity showed 95 per cent curl, *i.e.,* 95 per cent of the season's shoots showed curled leaves. This does not give a true picture of the severity of the disease, since in nearly every case every leaf of a shoot was curled.

It does not seem possible that the very few curled leaves in these orchards in 1926 could have produced ascospores and conidia sufficient to account for such an unprecedented amount of curl. The explanation must be that conidia are able to survive on twig surfaces longer than one year. This need not imply survival of individual conidia for so long a period, as saprophytic multiplication of conidia is quite possible.

Similar observations are reported by Young (32) and Anderson (1). Young describes an unusually severe outbreak of curl in Arkansas in 1926, following its marked rarity in 1925. Anderson describes the epidemic in Southern Illinois in 1926 as the worst he had ever seen, and states that curl had been almost completely absent there in the few preceding years.

Occurrence of Late Infections

The behavior of curl during a season following late spring infection has been described (16) as well as the fact that lesions due to late infection are small, separate areas scattered over the leaf blade. Such lesions are observable in many seasons when early infections also have occurred but are not always recognized.

Further evidence of late infections is obtained from observations of localized lesions on leaves some distance above the base of the season's shoot. The peach has indeterminate growth and not all the leaves of a season's shoot are present when the bud unfolds. There are usually 6 or 7 leaves present within the dormant bud. Small curl lesions on the blade of the leaf with no evidence of the disease on the petiole or adjacent part of the

shoot have often been observed on leaves arising from the ninth, tenth, eleventh, and twelfth nodes from the base of the shoot.

Waite (28) recently has described the occurrence in Alabama of an early curl infection involving the oldest leaves of a shoot and a later infection producing younger lesions on the tip leaves of shoots a foot or so long with from 7 to 11 healthy leaves below. Waite discusses the possibility of this late curl being due to secondary infection, but inclines rather to the belief that it may have been a case of delayed dormancy: *i.e.,* the fungus had not, because of an unusually mild winter, experienced enough cold to prepare it for spring germination. There is nothing in the life history of *Taphrina deformans* to suggest the necessity of a dormant period. In the experiments reported above infection resulted from inoculations made at intervals during the summer, fall, winter and spring. Inoculations of leaves just emerging from the buds with conidia that had been budding actively in culture for a year or more were quite as successful as those made earlier. The fungus clearly needs no rest period preliminary to infection.

It is probable that the early and late infections described by Waite could be definitely correlated with rain periods.

Secondary infections by the curl fungus may occasionally occur. Small, scattered lesions typical of late infections may sometimes be found on leaves so many internodes distant from the base of the twig that these leaves must have formed and become infected after ascospore dispersion from earlier infections. Such leaves are less likely to fall prematurely than those less recently infected. One such leaf has been observed on the tree as late as September.

The occurrence of late infections, whether primary or secondary, is of little practical significance. Spraying for curl is clearly an eradication measure, resulting in the destruction of conidia present on twig and bud surfaces, and if carefully done, it controls the disease. But growers may perhaps be made to realize that when their trees have escaped early infection there is still danger of curl that same season, and may thus be induced to spray every year.

PLACE OF HIBERNATION OF CONIDIA

The experimental results and observations reported here and earlier (15, 16, 17) together with the results of spraying experiments of Duggar (6), Pierce (19), and others point to the conclusion that bud conidia of *Taphrina deformans* pass the summer and winter on the surfaces of peach twigs and branches. Waite (28) states: "The spores . . . are supposed to lodge in the buds in late spring and early summer and to rest among the hairs in the bud scales during the winter. . . ." Wallace and Whetzel (29) state that circumstantial evidence indicates that spores are harbored

through the winter most probably on or between the bud scales. The same opinion is expressed elsewhere, as, for example, in Duggar's textbook: Fungous Diseases of Plants.

That the bud scales of the peach harbor a number of budding fungi has been shown by the writer (15), and the bud pubescence undoubtedly aids in retaining spores on bud scales once they have lodged there. At the time of ascospore dispersion, however, there are no peach buds with scales; only bud fundaments covered by the base of the petiole. Further, the bud conidia adhere to smooth surfaces. Cells from culture allowed to dry on glass wash away gradually under a spray of water, but some remain even after several hours' exposure.

There seems to be no reason for excluding any part of the surface of the peach tree as a place of hibernation.

Perennial Mycelium

Although it is clear that spring infection is traceable to overwintered bud conidia, this fact does not preclude the possible occurrence of perennial mycelium. Isolations from diseased twigs, undertaken to determine how long the mycelium survives in them, have already been reported (15), and additional isolations are described later in this paper. Success in such isolations does not depend, as was at first supposed, upon growth of mycelium from the diseased twig onto the agar. The method is, therefore, of no value in determining the point in question.

It becomes necessary, then, to rely on the observed occurrence of mycelium in twigs that have survived the disease and have made healthy growth beyond the hypertrophied portion, as described and figured by Duggar (6). Such twigs occur rarely in this locality and only on certain varieties. A few trees bearing them have been kept under observation and careful examination of the twigs was made in 1929, a season in which curl was early and was confined to the oldest leaves of the season's shoot.

In all cases the curl lesions involved the leaf only, sections failing to reveal mycelium in the stem of either the previous or the current year or even in the petiole. On the other hand, many curled shoots of the current year from branches that were healthy the year before showed hypertrophy of the petiole and adjacent portions of the stem. In such cases mycelium could be traced from the leaf through the petiole and down the stem to its point of disappearance. In no case did it extend more than a short distance down the twig of the current year. It is clear that, as in the curled shoots studied by Pierce (19), the mycelium had entered the stem from the leaf. A number of twigs that, this season, made healthy growth beyond the hypertrophied portion were sectioned and examined. A supplementary study was made of similar twigs collected in previous years and preserved

in Formalin or alcohol. Not only was the mycelium of *Taphrina deformans* absent from these twigs, but all the tissues formerly occupied by the fungus were dead. Healthy growth, resulting in extension of the twig, had been accomplished by tissues never occupied by the fungus.

From the peculiarities of *Taphrina deformans* when growing in culture, it is inconceivable that its mycelium can remain alive in dead host tissues, and the absence of mycelium from adjacent healthy tissues indicates the nonexistence of perennial mycelium. It has been shown earlier in this paper that the belief of various writers that *T. deformans* possesses perennial mycelium is based either on pure assumption, or on the imperfect observations of Sadebeck (25, 26). Unfortunately, the mere announcement of this belief has placed a burden of disproof upon subsequent investigators. It is proposed here to shift this burden and to state that, since there is no evidence whatever of the existence of perennial mycelium in *T. deformans,* it does not exist.

Complete study of the Taphrinaceae will undoubtedly reveal three types: forms with mycelium perennial in the branches, causing witches' brooms (*Taphrina cerasi*); forms with perennial mycelium in the buds, deforming whole shoot systems of the current season (*T. tosquinetii*); forms without perennial mycelium, affecting leaves (*T. aurea*); or leaves, fruits, and adjacent parts of twigs (*T. deformans, T. mirabilis*).

THE FUNGUS IN THE HOST: PENETRATION

Entrance of the fungus into the host has been observed in a few cases when conidia from culture were placed on detached leaves kept in moist Petri dishes. Portions of the leaf blades were then cleared and mounted. Other instances of penetration have been seen in fixed and stained sections of naturally infected leaves taken at frequent intervals, after the occurrence of prolonged spring rains, from heavily infected trees.

In all cases observed the fungus entered through the lower epidermis. Entrance through the upper epidermis, as reported by Fitzpatrick (8), undoubtedly occurs in the case of late infections but until the young leaf is well out of the bud it is folded along its midrib and the upper surfaces of the two halves are so held together by a waxy material that some difficulty is experienced in separating them. Only the lower surface of the leaf, therefore, is exposed.

The germ tube penetrates the epidermal wall (Fig. 1, A). Martin (14) states that she observed stomatal entrance, but the length of time (25 days) between inoculation and penetration, the fact that infection did not occur, and the lack of resemblance of the hyphae (figured approaching (not entering) the stoma) to mycelium of *Taphrina deformans* make her observation doubtful. Stomatal penetration may perhaps occur in late infections, but

in the infections of young leaves observed by the writer, it was impossible because the stomata were not yet developed. In sowings on older, detached leaves, spores often have been seen lodged in the stomatal entrance, but the growth of a germ tube into the stoma has not been observed.

In the youngest leaves in which penetration was observed there was no demonstrable cutinization of the epidermal wall, except directly beneath a large vein. The epidermal cells, however, contained numerous globules of a fatty material. In somewhat older leaves the fungus was seen penetrating a thinly cutinized epidermis. To what degree cutinization of the epidermal wall and the development of cuticle may act as a barrier to infection remains to be determined.

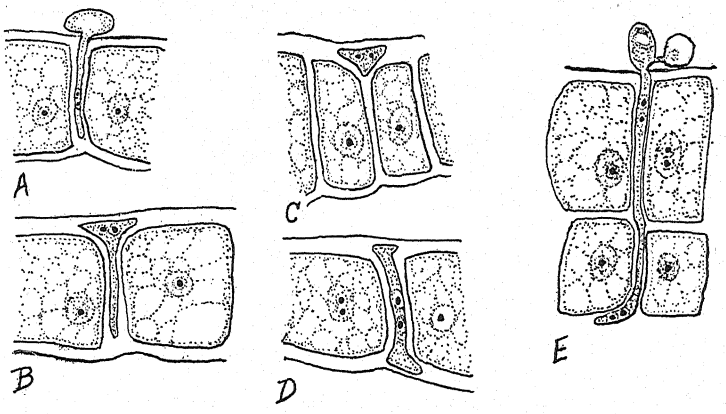


FIG. 1. Stages in penetration of the leaf by *Taphrina deformans*. A. Entrance hypha has penetrated epidermal wall and is growing in the radial wall between two cells of the epidermis. C. Subcuticular hypha, the spore is not visible. B, D. Subsequent growth of subcuticular hyphae in radial wall of epidermis. E. A case of penetration in which copulation of conidia apparently had preceded penetration.

After passing through the epidermal wall the penetration hypha often grows laterally, as reported by Fitzpatrick (8). Thus, stages in which the hypha is visible but not the spore itself are commonly seen (Fig. 1, C). The hypha now grows down between two adjacent epidermal cells (Fig. 1, B, D). This growth takes place perforce in the wall between the two cells. No intercellular spaces occur between the radial walls of the peach epidermis. Fitzpatrick (8), whose observations on penetration agree in general with those of the writer, is silent on this point, but one of his illustrations seems to show intercellular spaces between epidermal cells.

In all but one of observed penetrations the entrance hypha had arisen from a single conidium. In one case, however, (Fig. 1, E) copulation of conidia had apparently preceded emergence of the germ tube. One other instance of apparent copulation and subsequent growth of a tube from one

of the conidia has been seen on the surface of a cleared leaf; penetration, however, had not occurred. In the light of the findings presented below these observations are unusual.

SUBSEQUENT COURSE OF MYCELIUM

From the entrance hypha the mycelium extends at first (Fig. 2, A) between the lower epidermis and the first hypodermal layer (the spongy parenchyma not being yet differentiated). Subsequently, hyphae spread throughout the leaf, growing between the interior mesophyll cells. On reaching the upper epidermis, they spread out beneath it. This subepidermal mycelium is more abundantly developed than that beneath the

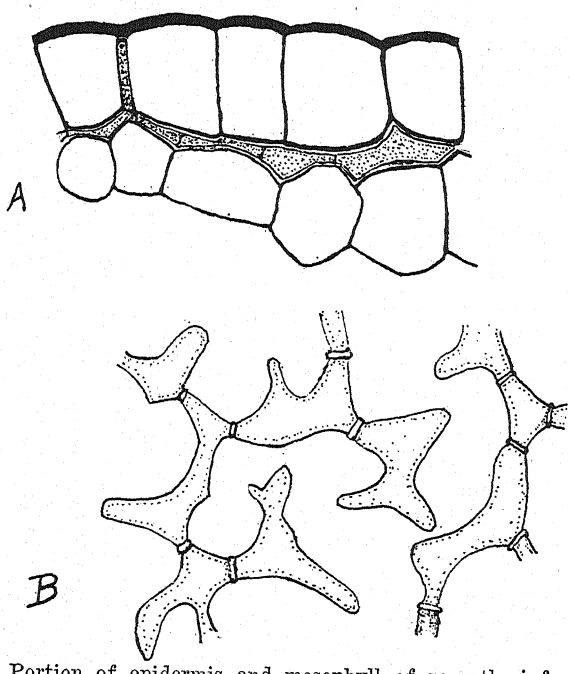


FIG. 2. A. Portion of epidermis and mesophyll of recently infected peach leaf, showing the subepidermal mycelium. B. Subepidermal mycelium as viewed from above in a cleared leaf. Host cells not shown.

lower epidermis. When viewed from above in cleared leaves, it appears as shown in figure 2, B. The irregular shape of these hyphae is apparently due to their fitting closely the intercellular spaces. The plate septa described by Pierce (19) may frequently be seen (Fig. 2, B). Judging from the appearance of these plate septa and from their observed swelling and dissolution in hyphae formed in culture they seem to arise as thickenings of the wall and to function as separation discs, allowing adjacent cells of the mycelium to separate from each other.

Frequently, in older much-curled leaves, the well-known tissue modifications are found only in the upper portions, involving upper epidermis, palisade parenchyma, and part of the spongy parenchyma, while the lowermost parenchyma and lower epidermis remain healthy and apparently free from mycelium. This suggests the possibility of entrance of the fungus through the upper epidermis, though it might conceivably result from the course of events described above together with more abundant development of the fungus in the upper part of the leaf.

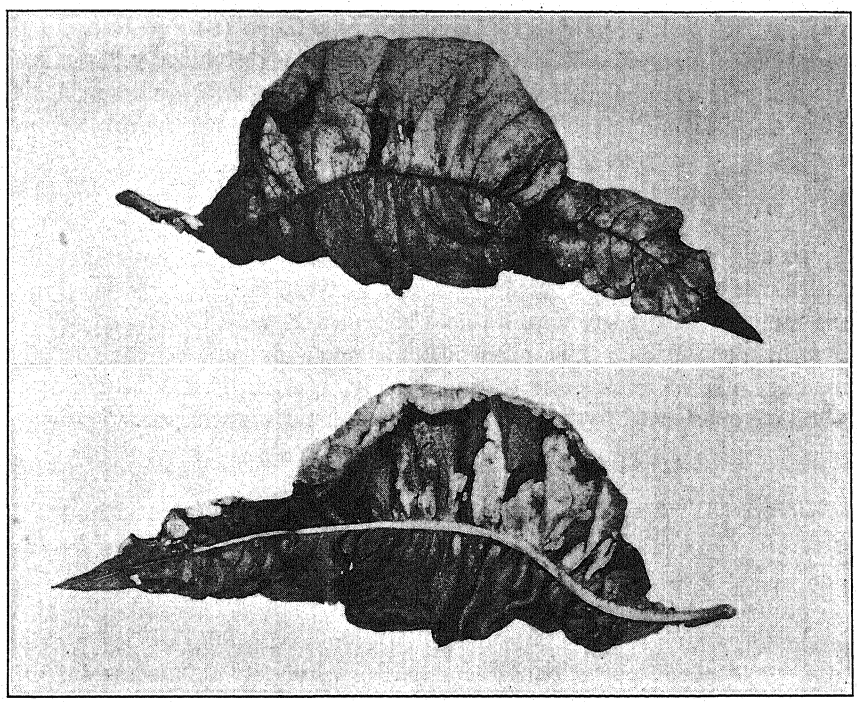


FIG. 3. A curled peach leaf that bore asci of *Taphrina deformans* on both its upper and lower surface. The whitish areas show the location of the hymenium.

From the mycelium beneath the upper epidermis, hyphae grow upward in radial epidermal walls. Branches of these hyphae form the subcuticular mycelium, ascogenous cells, and asci. A point of minor importance is the occasional occurrence of the hymenium beneath the cuticle of the lower epidermis. Winter (31) states that the asci break through the lower surface of the leaf, and this statement is denied by Knowles (12). Robinson (23) states that asci occur on both surface of the leaf but in greater numbers on the lower surface; Atkinson (2) finds them on both surfaces; while Pierce (19) dismisses these statements as having arisen "from a study of

other foliage than that of the peach and of other species of *Exoascus*. . . ." Asci do sometimes occur on both surfaces of the leaf in the case of badly curled leaves of susceptible varieties (Fig. 3).

CYTOLOGY

According to Dangeard (4), cells of the vegetative hyphae of *Taphrina deformans* are binucleate. Ikeno (9) describes them as multinucleate, Eftimiu (7) as binucleate or plurinucleate (12 to 14 nuclei). Eftimiu describes conjugate division of these nuclei. Fusion of the two nuclei in an ascogenous cell is reported by Dangeard (4), Ikeno (9) and Eftimiu (7). According to Ikeno and Eftimiu, the fusion nucleus divides by 3 successive divisions to form the nuclei for the 8 ascospores. Eftimiu identifies the first of these divisions as a reduction division, and reports the diploid number of chromosomes as 4, the haploid as 2.

Since the conidia are uninucleate, the manner in which the cells of the intercellular mycelium become binucleate has been uncertain. Eftimiu (7), having found binucleate conidia and germ tubes, decides that this condition is reached by nuclear division. Fitzpatrick (8), from observations on penetrating germ tubes and of tubes formed in culture, reaches the same conclusion. The penetration hyphae described above show in some cases one or more pairs of nuclei (Fig. 1, A, B, C, D), which apparently have originated by division of a single conidium nucleus.

COPULATION OF CONIDIA

Wieben (31) describes copulation of conidia and differentiation of the spores of one ascus into two sexes for *Taphrina epiphylla* Sadeb. and *T. klebahni* Wieben. The nucleus migrates from one conidium to the other. This binucleate conidium germinates by a long spiral thread. The tip of this thread, which may be cut off by septa from the older empty portions, contains cytoplasm and a pair of nuclei. Presumably this is the infection thread, and copulation of sexually different conidia may be a necessary preliminary to infection. Wieben's investigations, however, were not extended to determine this.[4]

In order to learn whether copulation of conidia may occur in the case of *Taphrina deformans,* cells from culture were placed on hanging drops of agar and observed from day to day under the microscope. In a few cases adjacent conidia put forth short papillae that joined to form a tube and one of the two conidia became progressively more vacuolate. Complete

[4] During a stay in Hamburg in 1930 the writer obtained a number of monosporous cultures of these fungi, but before the host (*Alnus incana*), which does not occur in Kansas, could be grown large enough for inoculation experiments, these cultures died from accidental exposure to high temperature. For the present this interesting question cannot be answered.

passage of the contents of one conidium into the other was not observed. In stained preparations cases were found showing apparent passage of a nucleus from one conidium to another and the subsequent emergence of a tube from the second conidium (Fig. 4). From other conidia long twisted



FIG. 4. Cases of apparent copulation of conidia seen in stained preparations from cultures of *Taphrina deformans*.

tubes bearing one or more pairs of nuclei were seen to arise (Fig. 5, F, G). These tubes, however, arose from single conidia, and it could not be determined whether copulation had preceded their formation.

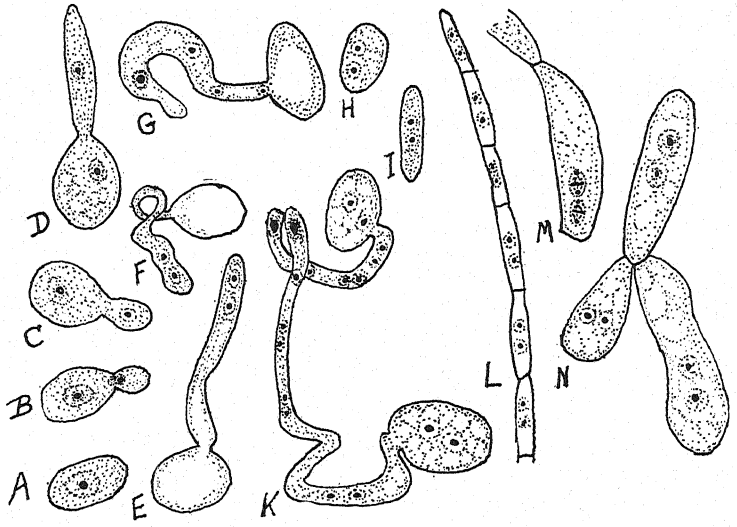


FIG. 5. Types of cells formed by *Taphrina deformans* in culture. A, B, C. Budding conidia. D. Conidium with straight, uninucleate tube. E, F, G. Conidia with irregular tubes bearing one or more pairs of nuclei. H, I. Binucleate conidia. K. Conidia with plurinucleate tubes joined at their tips. L, M, N. Hyphae with binucleate cells. M. Conjugate division in a binucleate hypha.

These observations were reported before the American Phytopathological Society in December 1928. In the abstract (18) of that paper it was stated for the sake of brevity that copulating conidia and binucleate germ tubes had been observed. In the light of subsequent findings it is regrettable that this statement was not fuller and more accurate.

The following summer a series of 8 monosporous cultures from a single ascus of *Taphrina deformans* was obtained. Asci were allowed to eject

their spores onto a film of agar on a cover slip.[5] An isolated group of 8 ascospores was chosen and the individual spores were separated by the Dickinson (5) method, using a micromanipulator devised for the purpose. From the resulting colonies transfers were made to agar slants and somewhat later cells from these cultures were placed on agar films and observed under the microscope. All possible pairings between the 8 cultures were made. Copulation observed was only occasional and incomplete (as described above) and there was no indication of sexual differentiation between the cells of different monosporous cultures. The experiment was repeated many times, using a number of different media, with no other results.

In the summer of 1930 the writer was privileged to work in Dr. Klebahn's laboratory in Hamburg and to repeat, with the assistance of Drs. Klebahn and Wieben, a part of the latter's work with *Taphrina epiphylla* and *T. klebahni*. Copulation of spores of these fungi was found to occur readily and exactly as described by Wieben (30). Difficulty was encountered in separating the 8 spores of an ascus because of the ejection of a quantity of sticky epiplasm along with the spores. Several incomplete series of 5, 6, or 7 monosporous cultures were obtained, but from lack of time no experiments were made on sexual differentiation.

Since, in the case of the fungi just mentioned, copulation is easily observed in the young colonies developing on agar films around a group of ejected spores, examination was made of such colonies of *Taphrina deformans* in this and subsequent seasons, but without result.

In *Taphrina epiphylla,* copulation occurs soon after conidia are formed, perhaps because infection occurs at once, and no such phenomenon can be observed in old single-ascus cultures. Further, when pairings were made in the fall of 1930 between cells from monosporous cultures some months old no copulation could be observed. On the other hand, the imperfect fusion of conidia observed in *T. deformans* has been found in only comparatively old cultures.

Further study of stained slides made from cultures of *Taphrina deformans* showed that the long twisted tubes arising from conidia are often uninucleate, or if they contain paired nuclei, they bear a single bright-staining nucleus close to the tip of the tube. Several cases were observed in which two such tubes from different conidia apparently were joined at their tips (Fig. 5, K). This suggested that copulation between conidia might have

[5] This method, or one in which the asci eject their spores upward onto agar in an inverted Petri dish, is the usual procedure in obtaining pure cultures of species of Taphrina. The 8 spores of an ascus form a close symmetrical group on the agar, easily located under the low power of the microscope. An ascus group is marked, the colony resulting from budding watched from day to day, and finally, tube cultures are made by transfer. Thus all cultures herein reported, except those from single spores, are from single asci.

occurred by means of such tubes. After much observation of conidia in agar films, one case was finally found where two such tubes came together at their tips and joined. Although kept under observation for several days there was no migration of protoplasm from one tube to the other nor any apparent alteration in the walls at the point of union. At the end of the period of observation the agar film was inverted, a cover slip added and pressure applied. The joined tubes could not be separated by pressure less than that necessary to crush the cells.

One example of apparent union of long tubes from conidia has been observed on the surface of a cleared leaf that had been inoculated and held in a Petri-dish moist chamber.

Inoculation Experiments with Monosporous and Single-ascus Cultures

The above observations indicated that copulation of conidia, as has been found in *Taphrina epiphylla,* does not occur with any regularity in *T. deformans.* However, the case of apparent copulation preceding penetration described in the foregoing, suggested that such copulation occasionally occurs. There was also the further possibility that union between germ tubes from conidia of opposite sex may occur after penetration of the leaf and that such union may be necessary to successful infection or to the formation of ascus-bearing mycelium. It was apparent that this possibility could be determined by inoculation experiments.

The first experiment was performed in 1930 in the Chelsea Physic Garden, London, on two potted yearling Peregrine peach trees furnished by the Department of Botany of the Royal College of Science. Inoculum from culture was inserted into the opening buds, and the high humidity of the greenhouse (an unheated fern house) made it possible to keep the buds wet for several hours. On April 1 one of these trees was inoculated with a single-ascus culture (163), the other with a monosporous culture (D4). On May 16 the first tree showed 20 curled shoots and 43 healthy. The second tree was dying, though it bore 30 shoots with leaves that were large enough to show curl. None of these leaves was curled.

Because of the inconclusive nature of this experiment inoculations were made in April, 1931, on young Elberta trees growing out of doors. These trees had been used for inoculation in the summer of 1929, but curled leaves borne in 1930 had been removed before ascospores were produced. The trees were sprayed with Formalin 1 to 100 a few days before inoculation. Shoots chosen for inoculation were some distance apart and so placed that the danger of splashing of inoculum from one to another was very slight. The fact that curl developed only on the inoculated shoots and on a few noninoculated ones close to some of them indicates that the experiment was trustworthy. Inoculations were made by the "smear method" at the begin-

ning of a rain period. Cultures used were Nos. D1 to D8, a series of monosporous cultures from one ascus, and Nos. 11, 176, 192, single-ascus cultures. Different shoots were inoculated with each monosporous culture, with each monosporous culture paired with every other, and with each single-ascus culture. Counts on May 18 (Table 4) showed curl resulting from every inoculation. All curled leaves produced ascospores.

TABLE 4.—*Results of inoculating peach shoots with monosporous cultures (D1 to D8) from one ascus, and with single-ascus cultures (11, 176, 192) of Taphrina deformans*

Branch number	Culture	Curled shoots	Healthy shoots
1	D1	8	17
2	D1 + D2	7	11
3	D1 + D3	13	14
4	D1 + D4	7	14
5	D1 + D5	15	27
6	D1 + D6	9	10
7	D1 + D7	11	14
8	D1 + D8	10	5
9	D2	10	12
10	D2 + D3	10	5
11	D2 + D4	3	16
12	D2 + D5	2	4
13	D2 + D6	10	11
14	D2 + D7	14	4
15	D2 + D8	11	16
16	D3	12	5
17	D3 + D4	11	9
18	D3 + D5	20	8
19	D3 + D6	16	20
20	D3 + D7	12	3
21	D3 + D8	9	18
22	D4	16	9
23	D4 + D5	8	10
24	D4 + D6	9	15
25	D4 + D7	13	18
26	D4 + D8	8	11
27	D5	8	10
28	D5 + D6	15	26
29	D5 + D7	8	8
30	D5 + D8	9	11
31	D6	5	15
32	D6 + D7	14	8
33	D6 + D8	13	6
34	D7	2	25
35	D7 + D8	4	7
36	D8	5	19
37	11	18	17
38	176	14	11
39	192	12	9

In connection with the above experiment it is worth recording that culture No. 11 was isolated in 1920. The fungus had, therefore, retained its virulence during 11 years of artificial culture.

Although the results of this experiment seemed perfectly conclusive, they were open to possible criticism, since the experiment was performed

out of doors. A second experiment, therefore, was planned, using young potted trees grown in the greenhouse and known to be free from curl. The performance of this experiment was delayed by lack of funds for the necessary equipment. Finally, in the spring of 1934, several such trees were inoculated with single-ascus cultures and others with monosporous cultures. These trees were then placed under a spray of water from a Tyler vapor head and kept there for several days. Curl did not result.

It was during the progress of this experiment that the paper by Fitzpatrick (8) was received in which successful inoculation of peach trees with monosporous cultures of the fungus is reported. Apparently, Fitzpatrick's arrangements to secure infection were better than those of the writer. All of the observations and experiments here reported were completed in 1931, and publication has been delayed only for this final confirmatory experiment. Under the circumstances, it does not seem worth while to repeat it.

Since infection resulting in typical curl can be effected by conidia descended from a single ascospore, it is clear that in *Taphrina deformans* copulation of conidia is not a necessary antecedent to infection, nor essential to the life history. However, in the case of penetration shown in figure 1, E, copulation had apparently preceded emergence of the germ tube, and this tube bore two pairs of nuclei. This observation together with that of imperfect cell unions and of joined tubes from different conidia reported above indicate that copulation does occasionally occur. This phenomenon may, perhaps, be reminiscent of a once functional sexual union, such as seems to occur regularly in *Taphrina epiphylla.* There is, of course, no direct evidence that copulation is a necessary antecedent to infection in the case of *T. epiphylla.* Further, Fitzpatrick (8) is in error when he states that the writer has postulated that copulation must precede the formation of a binucleate germ tube in the case of *T. deformans.* Clearly, the dicaryophase in this fungus is normally initiated by division of a nucleus of a single conidium, as stated by Eftimiu (7), and as shown by the observations on penetration made by both Fitzpatrick and the writer.

Nucleation of Cells Formed in Culture

The bud conidia formed in culture are usually uninucleate, and a new bud receives one daughter nucleus (Fig. 5, A, B, C). Short straight tubes put forth by conidia are usually uninucleate (Fig. 5, D). The long-twisted, tubes which sometimes join at their tips, are often binucleate or may show several pairs of nuclei (Fig. 5, E, F, G, K). In such cases the nucleus near the tip of a tube is more prominent and stains more brightly (Fig. 5, G, K). Occasionally, slender hyphae with binucleate, thin-walled cells are observed, as well as binucleate conidia (Fig. 5, L, H, I). In old cultures larger hyphae with binucleate, thick-walled or thin-walled cells are found (Fig. 5, N). Conjugate division of the nuclei of binucleate cells

has been observed, but the number of chromosomes could not be determined (Fig. 5, M). Rarely, plate septa have been seen between cells of thick-walled hyphae, and such cells have been seen to separate by dissolution of this septum. Fitzpatrick (8) states that the nuclei of tubes observed by him in culture are spaced far apart and do not suggest a true dicaryon. A dicaryon does occur, according to this author, in an infecting hypha. This seems a rather profound effect of the host upon a tube emerging from a conidium.

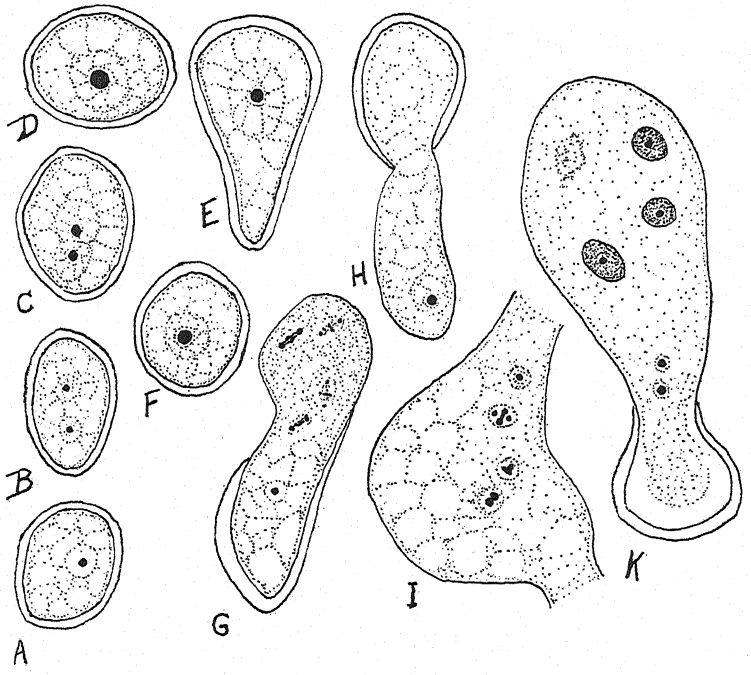


FIG. 6. Ascogenous cells and asci formed by *Taphrina deformans* in culture. A. Uninucleate; B. Binucleate ascogenous cells. C. Nuclear fusion in an ascogenous cell. D, E. Ascogenous cells with fusion nuclei. F. Ascogenous cell from hymenium on leaf to show comparative size. H. Young uninucleate ascus. G. Ascus with 5 nuclei, 4 of which are dividing. I. Portion of abnormally shaped ascus in which the nuclei contain brightly staining bodies, suggesting nuclear division in which the spindle fibers are not evident. K. Ascus with 3 spores, a degenerating nucleus, and two additional well-defined nuclei.

Thick-walled resting cells, previously described (14), may be uninucleate or binucleate (Fig. 6, A, B). Stages of nuclear fusion have been observed (Fig. 6, C, D) and cells with large, single, brightly staining nuclei that seem to be fusion nuclei (Fig. 6, D, E). The size and shape of these resting cells is variable, but often they resemble closely ascogenous cells found on the leaf (Fig. 6, F). It is believed that they are, in fact, ascogenous cells. Their mode of germination is additional evidence for this belief, and that

they have been observed to arise saprophytically in nature (14) is not evidence against it.

Resting cells germinate by rupture of the wall and the extrusion of a rather broad protoplast that may show contortions and twisting like that observed in tubes arising from conidia; in fact, it may occasionally be so narrow as to greatly resemble the latter. Some of these emergences are certainly asci. They bear a single prominent brightly staining nucleus (Fig. 6, H). The resting cell itself may at this stage show a degenerating nucleus, or the fusion nucleus may migrate to the young ascus. This appears to agree with the observation of Ikeno (9) who states that stalk cells are apparently cut off at different times, and that sometimes a nucleus ("chromatin bodies") remains in the stalk cell. Division figures of the fusion nucleus have been observed but the chromosomes could not be counted, nor could any synapsis stage be clearly seen, so that it cannot be definitely asserted that this is a reduction division. Asci with several nuclei, and with some of them in division have been observed (Fig. 6, G, I). One has been seen with 8 nuclei so well defined as to suggest that spore formation might be about to occur. Others have been found with 2 and 3 fully formed spores (Fig. 6, K). These asci formed in culture show some abnormality of shape, but they are comparable in size to those found in nature.

The significant part of these observations is that cells belonging to the dicaryophase and diplophase may develop in culture. It is further of interest that cells of all the types described above have been observed in monosporous, as well as in single-ascus, cultures.

Ascogenous cells and asci are found only in comparatively old cultures. While they have been formed on a variety of media, they occur more commonly in cultures on certain vegetable plugs, such as beet, carrot, and sweet potato.

ORIGIN OF THE SAPROPHYTIC PHASE OF THE LIFE HISTORY

All of the cultures of *Taphrina deformans* described by the writer (15), Klebahn (11), and Wieben (30) have been initiated by ascospores. An apparent exception occurred in the case of cultures isolated by the writer (15) by planting surface-sterilized pieces of curled leaves in tubes of potato-dextrose agar. It was at first believed that mycelium grew out from these pieces on to the agar. In continuing these isolations small pieces of leaf, approximately $\frac{1}{2} \times 1$ mm. were compared with larger pieces, 3 mm. $\times$ 5 mm. The small pieces soon turned dark brown and were evidently dead. No colonies developed from them. Most of the large pieces remained bright colored and apparently alive. By occasional transfer to fresh tubes of agar some of them were kept alive for twelve months. Around some of these pieces, colonies of *Taphrina deformans* developed from time to time.

In several cases the colony did not originate in contact with the leaf fragment but on the agar a few millimeters away. Microscopic examination of several tissue-fragments showed that the mycelium had remained alive in them and that asci and spores had formed on their surfaces. Successful cultures had apparently arisen from ascospores ejected onto the agar. It must be concluded that isolation from the interior of leaf tissues cannot be accomplished, and that cultures must be initiated by ascospores or conidia.

In nature *Taphrina deformans* is strictly parasitic in its diploid phase and saprophytic in its haploid phase. Saprophytic growth must be initiated by haploid cells, ascospores or conidia, but cells of the diploid phase may develop in culture.

SUMMARY

A review of the literature shows no convincing evidence that *Taphrina deformans* possesses a perennial mycelium. There is, however, evidence that conidia derived from ascospores by budding may survive and cause infection the following spring.

Attempts to isolate *Taphrina deformans* from the surfaces of healthy peach twigs were, as in the writer's earlier work, unsuccsesful.

Inoculation experiments have shown not only that conidia may infect the young unfolding leaves, but that they may do so after surviving on twig surfaces for more than a year. A bagging experiment has shown that, when ascospores are prevented from reaching twig surfaces, curl does not occur the following spring.

An experiment in summer spraying, planned to give evidence as to the survival of conidia on twig surfaces, was inconclusive. Field observations on peach leaf curl have shown that conidia may survive for more than one season and still be capable of infecting, and that late infections on well-expanded leaves may occur. There may even be a small amount of secondary infection in a season when early curl occurs.

Hibernation of conidia probably is not confined to the surface of bud scales, as stated by some writers. Conidia may be believed to survive on all surfaces of the dormant tree.

A study of the occurrence and distribution of mycelium in diseased peach twigs has shown that perennial mycelium does not exist.

Infecting conidia send germ tubes through the outer wall of the lower epidermis (perhaps, in the case of late infections, through the upper epidermis, as well). Some lateral growth may occur beneath the cuticle. A hypha then grows in the radial wall between two adjacent epidermal cells to establish the subepidermal intercellular mycelium. After some occupation of the interior intercellular-space system of the leaf, the mycelium forms a second subepidermal layer next to the upper epidermis. From this mycelium branches grow up in radial epidermal walls to give rise to

the subcuticular hymenium. Asci occasionally are borne on the lower, as well as the upper, surface of the leaf.

Evidence has been obtained from a study of cells in culture and of stained preparations of occasional copulation of conidia. Some evidence suggests that copulation between long irregular tubes from conidia may sometimes occur. A case of apparent copulation preceding penetration has been observed. Careful study of conidia from monosporous cultures has, however, shown no evidence of copulation between genetically different conidia. Wieben's observations as to copulation of conidia of *Taphrina epiphylla* and *T. klebahni* have been repeated and confirmed. The most that can be believed regarding copulation of conidia of *T. deformans* is that it is occasional and is perhaps reminiscent of a once functional process. The dicaryophase, in this fungus, normally originates by division of the nucleus of one conidium.

An inoculation experiment has shown that conidia descended from each of the eight ascospores of an ascus can infect. Copulation of conidia is therefore not a necessary antecedent to infection.

Incidental to this experiment was the fact that conidia may cause infection after being grown in culture for 11 years.

Cells of the dicaryophase and diplophase may be formed in culture. Such cells are: binucleate conidia, hyphae with binucleate cells (showing conjugate division of nuclei), ascogenous cells, and asci. Asci formed in culture are imperfect and often abortive, but a few have been found containing ascospores.

Although diploid cells may develop in culture, saprophytic growth (as evidenced by growth in culture) must be initiated by haploid cells, ascospores, or conidia.

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EXPERIMENTS ON THE CONTROL OF THE ROOT-KNOT NEMATODE IN THE FIELD WITH CHLOROPICRIN AND OTHER CHEMICALS[1]

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Concurrent with greenhouse and small-plot experiments on the use of chloropicrin and other chemicals for nematode control reported in another paper (7), 2 comprehensive field tests were installed, in the spring of 1931. Both were in Hawaiian pineapple lands known to be very heavily infested with the root-knot nematode, *Heterodera marioni* (Cornu) Goodey. Both were installed according to the Latin Square plot arrangement in order to reduce to the minimum the influence of soil heterogeneity in infestation. Undoubted value of chloropicrin for nematode control and for improvement of crop yields in nematode infested fields is indicated. Complete accounts of the experiments are given in this paper.

LITERATURE REVIEW

No attempt is made to review here all the extensive experimental work with chemicals for nematode control. As chloropicrin is of major interest, special literature studies were made on that chemical. It has for several years received the attention of entomologists, particularly as a fumigant for stored-product insects, but to some extent, also, for soil treatment. Roark (11) and Gersdorff (5) have supplied very complete bibliographies on all phases of the work with chloropicrin up to October, 1930. The work of Matthews (10) is significant of the possibilities with this particular chemical for nematode control, though her plot experiments with tomatoes were not highly promising in the matter of yield results. Her pot studies show striking benefits from the use of the chemical. Johnson and Godfrey (8), in row-treatment experiments, show striking results from chloropicrin applications, in reduced nematode population of the soil and improved pineapple yield. Godfrey, Oliveira, and Hoshino (7) report from 99 to 100 per cent reduction of nematodes where the chemical is adequately confined in the soil by gas-tight covers. Russell (12) reports good results in nematode control, and attributes remarkable root stimulation to the partial sterilization of the soil. He gives a number of illustrations showing this effect. Stone and Campbell (15) obtained good results with chloropicrin in controlling wireworms. Their use of it was as a contact applica-

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tion, in the form of an emulsion, rather than as a fumigant. Korab and Bootovsky (9) have recently reported tests with chloropicrin against the sugar-beet nematode, *Heterodera schachtii* Schmidt, with applications of 200, 400, and 800 liters per hectare. Increases in yield up to 500 per cent and of 2 per cent in sugar content were obtained. Four hundred liters per hectare (586 pounds per acre) is the ultimate rate of application recommended by them.

Several of the papers mentioned by Roark and Gersdorff (11, 5) and certain more recent papers indicate that chloropicrin has value as a soil fungicide and bactericide. Cooke (2) attributes apparent stimulatory effect of chloropicrin on sugar cane in Hawaii to its control of injurious soil fungi, particularly Pythium species, that attack certain varieties; Shchepetilnikova (13) reports it as an effective remedy against the fungi associated with flax sickness, at the same time augmenting the beneficial bacterial flora. Some of the improved plant growth resulting from experiments on the control of injurious soil fauna, therefore, may result in part from its effects on soil flora.

EXPERIMENTAL METHODS

The methods of preparation of the experimental areas, injection of chloropicrin, and subsequent procedure, were similar in the two experiments. The fields were plowed to the usual depth of about 12 inches (30 centimeters) and otherwise prepared for planting according to regular plantation practice. The specific areas for the experiments were then measured and the plots staked and labelled in accordance with detailed plans. The chemicals were injected into the soil in measured dosages, evenly spaced in the plant bed, by means of Vermorel carbon bisulphide injectors to depths of 6 to 8 inches. All beds were covered with the usual tar-impregnated pineapple mulching papers and the edges of the paper were covered to a depth of about 2 inches with soil. Differences in details of application between the 2 experiments are explained elsewhere. At safe periods after the treatments (not less than 48 hours) pineapple slips were planted through the papers originally used as covers, following regular field practice. No injury to the plants from the chemicals was evident at any time.

Measurements of the effectiveness of the treatments were then made in the following several different ways:

(1) Counts of numbers of Heterodera galls on the roots of cowpea indicator plants, planted in the pineapple plant beds for the purpose during the first month after treatment, after the method described in another paper (6).

(2) Thoroughgoing studies on random-selected pineapple plants removed with root system intact at 3 to 4 months after planting.

(3) Occasional photographs showing plant-growth differences between plots.

(4) Yield records at "plant crop" or first crop, harvested the second summer, from 15 to 18 months after planting.

The results throughout are analyzed statistically, according to the Latin Square system explained by Fisher (4) and Student (17), and significances of differences are determined by Student's tables (16) and Sheppard's tables (14).

Experiment 1.—A Latin Square Soil Fumigation Experiment with Chloropicrin at Different Rates of Application.

This experiment was installed in a Libby, McNeil, and Libby field in the Pupukea section, on the north-west slope of the Island of Oahu. The selected portion of the field had already been planted to pineapples the previous fall, but in February, 1931, it showed an extremely high percentage of failure and all plants examined showed heavy development of nematode root galls. Rather than replant irregularly, the plantation manager had the entire failing block of plants removed with a view to replanting throughout. The area was allotted to this experiment.

The planting plan in this section was the 4-row system, that is, 4 plant rows in the bed; the plants to be staggered, 15 inches apart in the row; plant beds covered with 54-inch-wide mulching paper; the beds spaced at 8-foot intervals, center to center, making about 17,800 plants to the acre. The area to be devoted to this experiment was about 40,000 square feet, or just short of 1 acre. This provided space for 36 plots of 5 beds each, with rows 27 feet long. These were arranged according to the Latin Square plan of random selection, as follows:

6	5	4	3	2	1	A plots, control, no treatment.
						B plots, control, no treatment.
F	B	E	A	C	D	C plots, chloropicrin, 130 pounds per acre.
A	D	F	C	E	B	D plots, chloropicrin 160 pounds per acre,
C	F	B	D	A	E	with extra cover (see text).
E	A	D	B	F	C	E plots, chloropicrin 160 pounds per acre.
B	E	C	F	D	A	F plots, chloropicrin 97 pounds per acre.
D	C	A	E	B	F	Each plot, 1,080 square feet.
						Total area, 39,000 square feet.

Certain irregularities in the shape of the field made for lack of complete perfection in the Latin Square arrangement, but these did not interfere with the legitimacy of the Latin Square system of analysis of results, and this paper is not complicated by the detailed explanation. Plot F in column 6 was smaller than the rest. This irregularity is provided for in the analysis of results.

Applications of treatments commenced on February 24. The applicators were adjusted to make the correct delivery per injection. It was

necessary, for some plots, to dilute the chloropicrin slightly with denatured alcohol or gasoline, so that each injection would deliver exactly the right quantity of chloropicrin. The basic rate of application, that used in F plots, was 2 cc. per hole in 3 lines of holes in each bed, spaced at 15-inch intervals, a total of 65 holes per bed, making 130 cubic centimeters per bed or 650 cubic centimeters per plot. On this basis the rate of application was 97 pounds per acre (approximately 54 liters, or 90 kilograms per hectare). Plots D and E, receiving the heaviest applications, received exactly the same amount per injection but with 2 additional lines of holes along the edge of the mulching paper. This gave a rate of application 2/3 greater than the basic rate, or approximately 160 pounds per acre (150 kilograms per hectare). The C plots received 1/3 more chloropicrin per injection spaced as in F plots, making about 130 pounds per acre (120 kilograms per hectare).

The action of the chemical upon the valves of the applicators made them somewhat erratic in their delivery, so that constant checks upon the quantities delivered had to be made. These quantities varied sometimes in different plots of the same treatment, usually within 5 or 10 per cent of the desired, but as high as 25 per cent off in 1 or 2 beds. Unfortunate as this was in its tendency to bring about irregularities in results without opportunity for making due allowance in calculations, it was unavoidable with the equipment available. Detailed notes made for each plot show actual applications to be slightly higher on the average than the desired quantity. It is certain, however, that the relative relationships of quantities used in plots C, D, E, and F were very close to the ratios planned.

Since, through misunderstanding, the mulching paper was already laid by the plantation men in preparation for regular planting at the time planned for application of the chemical, it was necessary to insert the chloropicrin into the soil through holes punched through the paper with the applicators. A second man with each applicator covered the holes with earth, and the soil at each point was firmly compacted with the foot. This prevented quick escape of the gas through the hole, but did not permit of the degree of retention that might have been attained had unbroken covers been used. Correct 15-inch spacing of points of application was attained by the use of marked planting lines, the same ones that were used later for planting the pineapples in the same beds. Extra covers, extending from near the centers of beds to about 6 inches from the centers of "middles" between beds, were placed over D plots. This was designed to bring about better retention of the gas in the soil. The extra covers were removed before pineapples were planted. The operation of installation was delayed by a period of intermittent showers until March 5, when final beds were treated. At no time were applications made in soil that was nearly satu-

rated with moisture, but only in soil of loose loamy texture. Pineapples were planted within a few days after treatment. A line of plants was omitted between plots in the "columns" (see planting plan) to permit of sharp plot demarcation. Figure 1 is an aerial view of the experimental area taken about 11 months after planting.

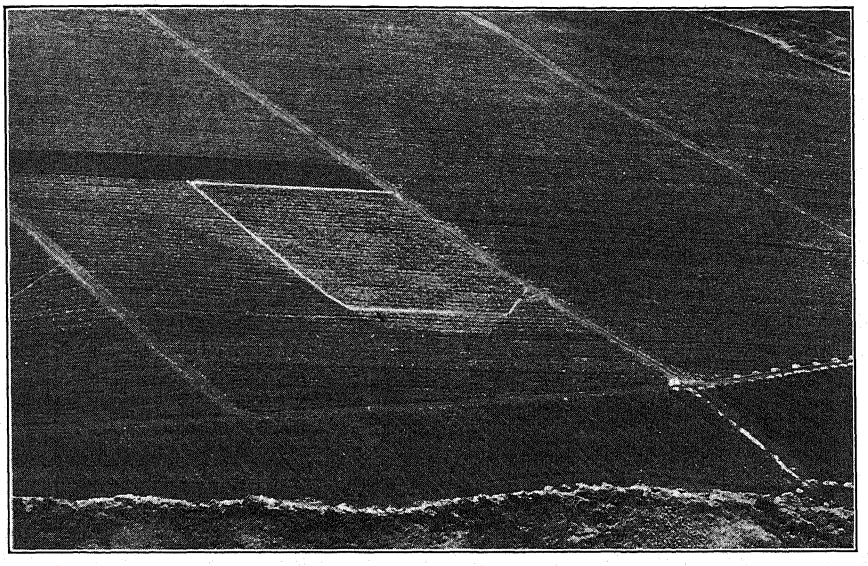


FIG. 1. Airplane view of the area used for experiment 1, marked with fine white lines. The road to the right of the area corresponds to the base line of the layout plan shown on page 78. The plots are in the shape of parallelograms rather than rectangles. In the original photograph there is evident a faint, irregular, checker-board effect, brought about by the contrast between treated and control plots, which can be identified by referring to the before-mentioned layout. The dark line in the lower left corner of the experimental area is the shadow of the airplane from which the picture was taken. Photographed by the 11th Photo Section, Air Corps, Luke Field, T. H., March 10, 1932, about 11 months after the pineapples were planted.

RESULTS FROM EXPERIMENT 1

Within 3 months after planting distinct differences became evident in vigor of plant growth, between treated and nontreated plots. At from 3 to 5 months the relative under-size in nontreated plots was accompanied by chlorosis, a distinct yellowing, as compared with the treated plots. Evidently, reduced root development in nontreated areas made for a reduction in iron absorption. Sprayings with iron sulphate according to regular plantation practice so overcame this difference that, after 6 months, color differences were less pronounced. Airplane pictures, taken during the first year of growth, at 5 and 11 months after planting, through the courtesy of the 11th Photo Section, Air Corps, Luke Field, T. H., show fairly well

such plot differentiation. Figure 1 is a reproduction of one of the later photographs. Plot differences in size of plants increased until near the time of fruit production and striking differences persisted throughout the life of the plants, similar to those shown by Johnson and Godfrey (8) in their row-treatment experiments. Figure 2 shows the adjacent lines of plot 1–D, right, and nontreated control, left, with size differences strikingly evident, at 12 months after planting. Certain weeds, particularly the "popolo" (*Solanum nodiflorum* Von Jacquin), growing in the pineapple rows, likewise showed striking differences in growth. When pulled, such weeds showed outstanding differences in amount of root-gall development, but no quantitative records were made by which such differences can be presented. Quantitative measurements of the effects of treatments in this experiment consisted of root-gall count on indicator plants, grown at the

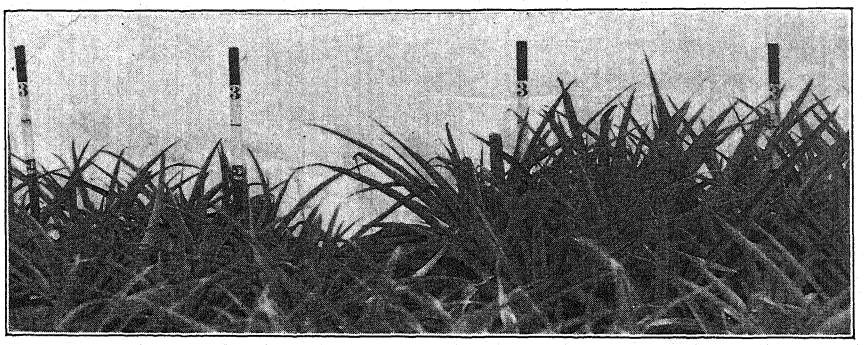


FIG. 2. Photograph, taken about a year after planting, showing the striking difference in size of pineapple plants between a nontreated area, left, and a chloropicrin-treated area (1–D) right. The marked stakes are flush with the ground level at the zero point. Treated plants are over 3 feet high, while nontreated are barely over 2 feet.

edges of the paper and in holes cut through the paper with hoes, and of yield records.

Results in Root-gall Counts on Indicator Plants.—In order to obtain an earlier quantitative estimate of the effects of the treatments than could be obtained from observations on pineapple plants, Whippoorwill cowpeas were planted shortly after pineapple planting, in holes cut through the mulching paper, in the middle 3 beds of every plot. Moisture conditions were favorable for sprouting, and plant growth was good. The plants were removed, after about 1 month of growth, with root systems as complete as was possible, by first loosening the plant with a trowel and pulling gently on the top. Since entire root systems were not obtained, considerable irregularity in root volume removed was unavoidable. It is obvious, therefore, that no high degree of accuracy in nematode-population estimates could be expected. Where great differences occurred because of

treatments, such differences might be expected to appear in gall-count readings. Smaller differences, such as those between treatments, might not be expected to be indicated so satisfactorily by this rough method. Actual numbers of plants per plot examined ranged mostly between 300 and 600.

For purposes of comparisons between plots the direct readings in terms of root-gall average counts, by plots, was considered satisfactory without multiplication by a constant factor, as suggested elsewhere (6), for transforming the results to an approximation to actual soil infestation. In order to eliminate large decimals in the analysis of results, readings are analyzed in terms of numbers of galls per 100 plants. Without complicating this paper by the details of preliminary calculations, the analysis of readings by Fisher's Latin Square method resulted in a table of variances, as follows:

TABLE 1.—*Table of variance in root-gall count per 100 nematode-indicator cowpea plants*

Variance due to	Degrees of freedom	Sums of squares	Variance	Standard deviation
Treatments	5	10383759.18		
Columns	5	1318579.53		
Rows	5	1308133.78		
Remainder	20	6144866.13	307243.30	554.3
Total	35	19155338.62		

As is to be expected from the rough method of measurement, the standard deviation is very high, so high, in fact, as to appear unfair to the more even distributions of the better treatments. (The standard deviation of the distribution of readings by the normal curve method, on D plots, for example, is only 66.3, while that on E plots is 102.5.) Continuing with the Latin Square analysis, however, 554.3 is multiplied by the factor $\frac{2\times 21}{6}$ to obtain 1466.6 with which plot differences are divided to obtain "Z," used in Student's tables.

The differences between plots in gall counts, and percentages, and the significances of differences are shown in table 2. The average gall count in A and B check plots together is 1240.4. The P values are tabulated as the criterion of significance. Since a P value of .997 is equivalent to a $\frac{D}{PE}$ of 4, P values of table 2 of .997 or higher are thoroughly acceptable assurance that differences are due to treatments and not to chance, while a P value only slightly less is still very highly significant. It is to be seen that, even with the rough measurement used (gall count on roughly removed plants) with very high standard deviation, reductions in nematode

TABLE 2.—*Comparisons of plot treatments in root-gall counts per 100 nematode-indicator cowpea plants, with significances of differences*

Plot	Treatment (chloropicrin, lbs.)	Gall count	Comparison with	Difference	Percentage difference	P	Significance (odds)
$\frac{A+B}{2}$	0	1240.4	.....	.....	.....	.....	.....
C	130	67.5	Checks	1172.8	94.5	.9993	1428 to 1
D	160	69.0	"	1170.5	94.4	.9987	768 to 1
E	160	69.3	"	1171.2	94.4	.9988	832 to 1
F	97	433.9	"	806.5	65.0	.9862	72 to 1
E	160	69.3	F	364.6	84.0	.8489	16.6 to 1

count of better than 94 per cent occur with the 3 higher treatments, with certainty of significance, while with only 97 pounds of chloropicrin per acre 65 per cent reduction occurs with odds 72 to 1 in favor of significance. The rough measurement did not serve to show any significant difference between 130 and 160 pounds per acre, and the difference between the 97- and the 160 pound treatments, while a large one, is not highly significant statistically. The measurement of differences in plot yields, made with much greater accuracy, shows plot differences of higher significances.

Results in Differences in Total Plot Yields.—Beginning in June, 1932, and continuing into September, during the normal ripening period of the pineapple crop, yield records were taken on all plots. Accumulated totals of numbers of plants harvested and of yields were taken without effort to obtain individual plant records. Unfortunately, one error was made when, through misunderstanding, the regular plantation-field harvesting crew began to pick fruits and removed a portion of those from plots B and D in column 6. This removed the possibility of complete accuracy in total yield figures for these plots. In order to obtain a figure for purposes of calculation, the same values are allotted to these plots as was calculated accurately for the average of the other 5 plots of the same treatments. These plots were not outstandingly different from the average, and in all probability this figure is not far from the correct one. The same sort of adjustment

TABLE 3.—*Table of variances in total plot yields, in pounds of pineapples*

Variance due to	Degrees of freedom	Sums of squares	Variance	Standard deviation
Treatments	5	649032		
Columns	5	122609		
Rows	5	57293		
Remainder	20	120328	6016.4	77.56
Total	35	949262		

was made for plot F in column 6, since, as stated in the introduction to the experiment, this plot was considerably smaller than the others.

Table 3 shows the yield-data variances from which the standard deviation due to random error is calculated.

Multiplying 77.56, the standard deviation due to random error, by the factor $\frac{2 \times 21}{6}$, the constant 189.94 is obtained, with which plot differences are divided to obtain Z, used in Student's tables. The differences between plots in yields per plot and the percentage differences, together with significances, are shown in table 4. The average yield per plot of A and B plots together, taken as before for purposes of comparison, is 1022 pounds.

TABLE 4.—*Comparisons of plot treatments in yield per plot, in pounds of pineapples*

Plot	Treatment. Chloropicrin (pounds)	Yield pounds	Comparison with	Difference	Percentage increase	P	Significance (odds)
Checks	.....	1022	.....	.....	.....	.....	.....
C	130	1286	Checks	264	25.8	.9999	10000 to 1
D[a]	160	1327	"	305	30.0	.9999	"
E	160	1343	"	321	31.4	.9999	"
F	97	1164	"	142	13.9	.9980	500 to 1
E	160	1343	C	57	4.4	.9026	9.3 to 1
E	160	1343	F	179	15.4	.9997	3332 to 1
C	130	1286	F	122	10.5	.9953	212 to 1

[a] With extra cover for better confinement of the gas. See text.

All treatments, including even the very low one of only 97 pounds of chloropicrin per acre, showed undoubted increase in yields, the maximum being better than 31 per cent with one of the 160 pounds per acre applications. No difference between results from the 2 methods of application of D and E plots is evident, so the additional cost of an extra cover is not indicated. The 160-pound application was not significantly higher than the 130 pound rate, in results. Both the 160- and the 130-pound applications, however, were definite improvements over the 97-pound treatment. These results are subject, of course, to the slight error introduced by the uncertainty as to exact total yields in B and D plots in 1 series.

Still another analysis of yield results was made, on the basis of average weight per fruit. This figure was obtained as a result, by plots, of total yield actually harvested divided by total number of fruits harvested. The results are shown in table 5.

A complete Latin Square analysis of results, on the basis of even numbers of plants, was made. The standard deviation due to random error, as

TABLE 5.—*Average pineapple fruit weights, by treatments*

Plot	Treatment	Average number of fruits	Average weight per fruit (pounds)		Critical difference, 0.188
A	Control	342	2.99	3.01	
B	"	326	3.03		
C	130	345	3.74		
D	160	338	3.86		
E	160	356	3.74		
F	97	296	3.51		

calculated, is .103. The constant, for the determination of Z is .2725. For the sake of simplicity in making comparisons, the "critical difference" was determined, at or above which significance is completely reliable, with odds of 332 to 1 or higher. As shown in the table, this figure is 0.188 pounds. It is thus to be seen at a glance that all the treatments gave significantly higher yields per plant than the average of the checks. Likewise, C, D, and E show significantly higher averages than F. Other differences are not significant.

Experiment 2.—A Latin Square Soil Fumigation Experiment with Chloropicrin and Carbon Bisulphide.

This experiment was installed shortly after experiment 1 in a field in the heart of the best pineapple area in the interior of the island of Oahu and known to be very heavily infested with nematodes. The area selected, about 1 acre, had been planted to an experimental block of *Sesbania macrocarpa* Mühl., a highly susceptible legume, and it is certain that heavy reproduction of nematodes had occurred. This had been plowed under within the previous few months, and the field prepared for pineapple planting, according to regular practice.

The layout and installation of the experiment were similar to those of experiment 1, with differences as follows: The system of planting was a 2-row system, 2 plants in a cluster, with 20-inch spacing, beds 6 feet center to center, making approximately 17,000 plants per acre. Each plot consisted of 6 beds each 25 feet long, a total of 900 square feet or approximately 1/50 of an acre—a convenient figure for calculating quantities of chemicals needed per plot and bed. Each bed contained 30 clusters of plants.

The chemicals were applied with the Vermorel injectors used in experiment 1, in 2 lines of holes with 10-inch spacing, or just half that of the

pineapples, thus making for good general diffusion of the gases through the soil. Plots D and F of the chloropicrin treatments (see layout of experiment, to follow) received an extra line of treatments at either edge of the permanent mulching paper, directly opposite the plant clusters. Carbon bisulphide plots, receiving 900 cc. per plant bed, received 3 injections of 5 cc. each, in 60 different spots. Thus each planting point was well surrounded with injections. The applicators were adjusted for correct delivery, but unavoidable discrepancies, mostly in the direction of slight excess occurred. These are disregarded in calculations, throughout, inasmuch as relative proportions are very close to the desired.

The applications were made directly in the ground and the injection hole pressed with the foot to prevent too rapid immediate evaporation. Immediately after the completion of each bed of a block, mulching paper was unrolled to cover the bed and the edges were covered with soil. This gave better confinement of the gas than was obtained in experiment 1, in which the holes punched through the paper permitted of more rapid escape. In accordance with previous experience, recorded elsewhere (7), a higher degree of efficiency might be expected from this better confinement. In plots F and G (see layout) 2 extra strips of paper were laid on each bed extending approximately to the middles (between beds) for the purpose of still further contributing to the efficiency of gas confinement.

There were 7 treatments, including 2 controls, each replicated 7 times. The plots were arranged according to the Latin Square plan of random selection, as follows:

7	6	5	4	3	2	1
D	C	B	E	G	F	A
B	E	G	C	D	A	F
F	D	C	A	E	B	G
G	A	D	F	C	E	B
E	F	A	G	B	C	D
C	B	F	D	A	G	E
A	G	E	B	F	D	C

Each plot consists of 6 beds.
Beds 25 feet long, 6 feet center to center.
Area of each plot, 900 square feet.
60 plants to each bed, 360 to the plot.
Total area, 44,100 square feet.

Treatments were as follows: A, control, nontreated, except for usual mulching paper cover; B, chloropicrin 150 pounds per acre; C, carbon bisulphide, 750 pounds per acre; D, chloropicrin, 170 pounds per acre; E, control, identical with A; F, chloropicrin 170 pounds, with extra cover; G, carbon bisulphide 750 pounds, with extra cover. The position and surroundings of the experimental area are shown in figure 3, an airplane view, taken from approximately 700 feet about 5 months after planting.

Shortly after the treatments, pineapple slips were planted through the mulching paper according to regular plantation practice. Thereafter, in general, regular field cultivations along the rows were avoided in order to

keep to a minimum the spread of infestation from one plot to another. Unfortunately, through misunderstanding as to responsibility, such cultivation occurred at least once. Weeds were removed by hand or by hoes. Volunteer Sesbania plants developed in considerable abundance throughout the experimental area and were treated as weeds. Occasional observations on such plants disclosed undoubted effectiveness of treatments in reducing soil infestation, for those in the control areas were as a rule very heavily infested, while those in treated plots were frequently quite free from galls.

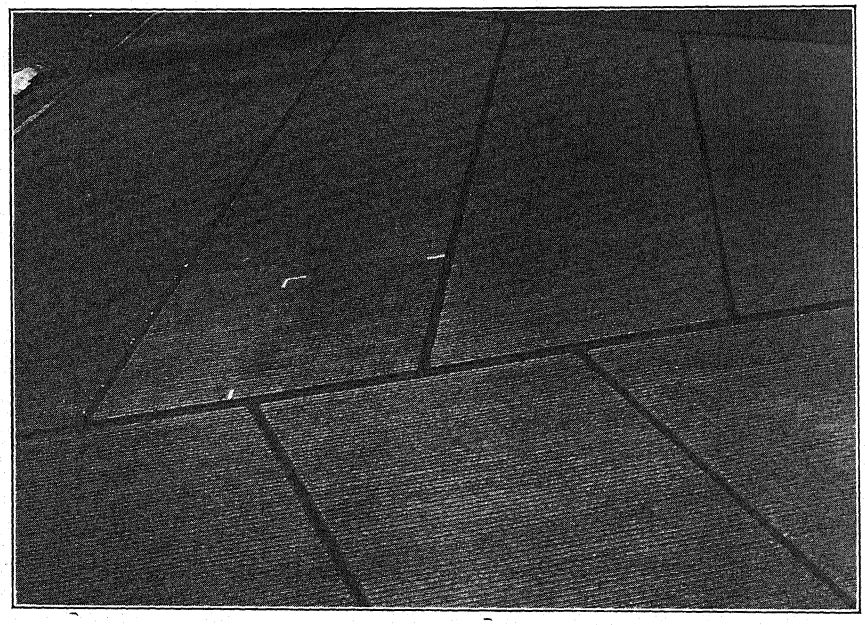


FIG. 3. Airplane view of the area used for experiment 2, 5 months after planting, 3 of the corners being marked with white strips of cloth. The road on the right of the area is the head line corresponding to that of the layout shown on page 71, column 1 being on the nearer side of the picture. Contrasts between nontreated controls and the better treatments are clearly distinguishable in the original photograph. Vacant spaces left by the removal of plants for detailed measurements, are clearly shown. The photograph was made possible through the courtesy of the 11th Photo Section, Air Corps, Luke Field, T. H.

No quantitative studies were recorded on such plants, however. Particular care was taken throughout the growth of the pineapples, up to plant-crop stage of development, to prevent, by spraying with oil emulsion as frequently as necessary, the development of mealy-bug infestations, whose effects in producing "mealy-bug wilt," as reported by Carter (1), would seriously vitiate the uniformity of nematode-control results.

As with experiment 1, within 3 months after planting striking differences between treatments became evident, in general vigor of plant growth,

as indicated by an unhealthy yellowish-green color, as well as by dwarfed growth. Figure 3 shows the checkerboard effect to some extent, but not nearly so strikingly as it actually appeared, because of lack of good color contrast in the photograph. Figure 4 shows comparative sizes of the adjacent rows of plots 5–E, left, and 4–B, right, on April 14, 1932, 12 months after planting. Reference to the layout plan and perhaps to figure 3 will determine exactly where the picture was taken.

Results of the treatments were measured by 3 separate studies, as follows:

(1) Counts of galls on cowpeas, planted as nematode indicator plants.

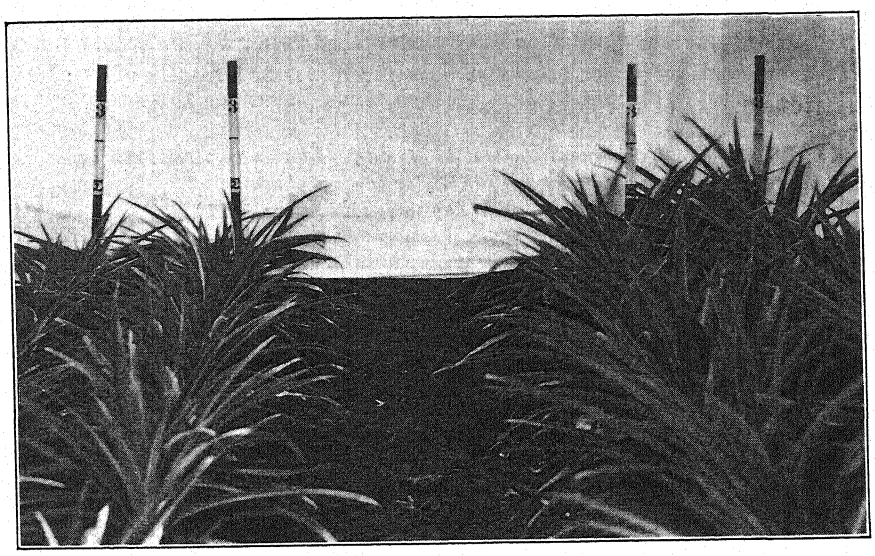


FIG. 4. Photograph to show size differences between nontreated and treated plots in experiment 2, at about a year after planting. Left, plot 5–E, control; right, 4–B, chloropicrin 150 pounds to the acre. Note that the treated plants are about a foot taller than the nontreated.

(2) Measurements and gall counts on roots, and weights of tops, of 16 pineapple plants per plot, removed from the middle 4 beds of every plot from 3 to 4 months after planting.

(3) Complete plant-by-plant yield records made at plant-crop harvest during the summer of 1932.

It had originally been planned to go still farther into the analysis of results with yield records and, immediately afterward, complete root studies on representative random-selected plants from every plot, for the purpose of calculating correlations between root condition and yield on the same plants. Circumstances, however, prevented the consummation of these plans.

Results in Root-gall Count on Indicator Plants.—Whippoorwill cowpeas were planted through holes in the mulching paper, between the pineapple rows and at the edge of the paper, shortly after the pineapple plants were set and before the ground became shaded by them. The stand was somewhat uneven, but always the numbers were sufficient to provide good plot readings. The plants were removed when about a month old, somewhat roughly, as in experiment 1, but permitting of gall-count classifications, by treatments, with highly significant differences. The numbers of plants upon which observations were made were somewhat variable, but, as a rule, there were well over 100 per plot. Results were reduced to average numbers of galls per plant for each plot, and, in order to eliminate large decimals, these figures are reported as numbers of galls per 100 indicator plants.

For the sake of space economy, only the important results of the Latin Square analyses of the data are recorded, as shown in table 6.

TABLE 6.—*Comparisons of the results of soil-fumigation treatments in experiment 2, in root-gall count per 100 nematode-indicator cowpea plants*

(*Standard deviation due to random error,* 1372
Critical difference, 2262)

Plot	Treatment pounds per A.	Gall count	Comparison with	Difference	Percentage reduction
Checks	.....	10741	.....	.....	.....
B	Chloropicrin 150	1810	Checks	8931	83.1
C	Carbon bisulphide 750	6445	"	4296	40.0
D	Chloropicrin 170	1297	"	9444	88.0
F[a]	Chloropicrin 170	831	"	9910	92.2
G[a]	Carbon bisulphide 750	4660	"	6081	56.6
G			C	4635	80.0
B			C	1785	27.7

[a] Extra covers were used over these plots; see text.

Even though the standard deviation due to random error is rather high, all differences shown—that is, all treatments compared with checks, and all chloropicrin treatments compared with carbon bisulphide—are highly significant. The final comparison shown in the table, carbon bisulphide with and without the extra cover, shows considerable difference, with the odds 65 to 1 that the difference is due to the difference in treatment rather than to chance. The differences between chloropicrin treatments are not significant. The additional cover used in F was not of any significant value. The effects of the reductions that were brought about by the treatments in nematode population, on gall count on the roots of the planting of pineapples, and on root and top development of that crop, are shown in the next section of the paper.

Results in Readings on Random-selected Pineapple Plants.—The plants for this study were selected by marking with stakes the 5th and 10th hills of 2 plants each in the middle 4 beds of every plot, half of them on the east and half on the west row of the bed. Thus 16 plants were taken from each plot, a total of 784 plants from the entire experimental area. Beginning in July, 1931, about 4 months after planting, plants were carefully excavated with root systems intact, a very painstaking operation, requiring over a month for completion. The order of removal was such as to distribute evenly, by treatments, the factor of the influence of time on growth; this factor, therefore, can be ignored. The plants were taken to the Honolulu laboratory, where root systems were washed and the following complete records were taken:

Total number of roots per plant, counting as such only the main large roots, arising directly on the planting piece; total length of root system per plant, obtained by adding the lengths of all main roots counted; total volume of root system per plant; total number of terminal nematode root galls per plant; total number of non-terminal nematode root galls per plant; total number, terminal and non-terminal galls together; total number of small galls on lateral rootlets; total number of prominent *Tylenchus brachyurus* lesions; total number of roots less than 6 inches in length; total number of roots 12 inches or more in length; total weight of plant, exclusive of roots.

The results, in averages only and by treatments, of these various studies are given in compact form in table 7. For the sake of ready visualization of the treatment differences, the plots are arranged in the table with the check-plot average first, followed by the carbon bisulphide treatments, and finally the chloropicrin treatments. In the table the various features measured are lettered for ready reference for correlation studies that appear later. Item "a" consists of the earlier derived data on indicator-plant, root-gall readings. Items "m" and "n" consist of the yield data brought forward from the next section of this paper for convenient comparison with the other data.

The rank, in order of efficiency of treatments, is obvious in most cases. With greatest efficiency one finds (a) the lowest root gall counts; the highest figures for length and volume of roots (c and d); the lowest counts in e, f, g, h, and i; the lowest count in numbers of short roots (j) and the highest in long roots (k); the highest weights (l); and naturally the highest in yield records, (m and n). For number of roots (b) alone there is an apparent inconsistency with other results, though with significantly lower numbers in treatments C, G, D, and F than in the checks, there would appear to be a definite proliferation of unnatural early root development in the plants that were heavily parasitized by nematodes, similar to the prolif-

TABLE 7.—*Plot averages of readings on various bases of comparisons on nematode-indicator cowpea plants and pineapple plants to show results of treatments in experiment 2. The last column gives, for some cases, the minimum plot difference that indicates unquestionable significance. Significant differences are indicated, as per footnote. The bold-face superior numbers, indicate the rank of the treatments in order of effectiveness*

Designation	Basis of comparison	Controls (average)	Treatments					Critical difference and significant[a] differences
			Carbon bisulphide		Chloropicrin			
			C	G	B	D	F	
a	Indicator-crop gall count	**6** 107.4	**5** 64.5	**4** 46.6	**3** 18.1	**2** 13.0	**1** 8.3	22.5 s-1, -2, -3
Young pineapple plants								
b	Number of roots	**1** 34.3 ± .43	**3** 31.2 ± .68	**5** 30.1 ± .71	**2** 32.6 ± 1.06	**4** 30.7 ± .69	**6** 28.9 ± .91	s-1 ex. B, -2
c	Length of roots	**6** 291.5	**5** 305.8	**4** 312.4	**2** 387.0	**1** 392.1	**3** 356.1	60.34 s-1, s-3 ex. F
d	Volume of roots, cc.	**6** 25.5 ± .71	**5** 25.5 ± .56	**4** 26.1 ± 1.08	**2** 30.8 ± .59	**1** 32.8 ± 1.01	**3** 30.2 ± 1.24	s-1
e	Number of terminal galls	**6** 12.8	**5** 7.7	**4** 6.2	**3** 5.3	**1** 3.1	**2** 3.2	3.16 s-1, -2, -3 ex. B
f	Nonterminal galls	**6** 2.7 ± .15	**5** 1.5 ± .20	**4** .9 ± .11	**1** .27 ± .03	**3** .46 ± .03	**2** .30 ± .11	s-1, -2
g	Total galls	**6** 15.6 ± .66	**5** 9.2 ± .29	**4** 7.1 ± .53	**3** 5.8 ± .64	**2** 3.9 ± .09	**1** 3.2 ± .28	s-1, -2, -3

TABLE 7.—(*Continued*)

Designation	Basis of comparison	Controls (average)	Treatments					Critical difference and significant[a] differences
			Carbon bisulphide		Chloropicrin			
			C	G	B	D	F	
		6	5	4	1	3	2	
h	Lateral rootlet galls	29.0	6.0	11.0	2.8	3.6	3.4	s-1, -2, -3
		6	5	3	4	1	2	
i	Tylenchus lesions	10.5 ± .33	10.3 ± .33	8.2 ± .53	9.9 ± .28	6.3 ± .39	6.8 ± .46	s-1, ex. B
		6	5	4	3	1	2	
j	Number, roots < 6 inches	13.9 ± .26	11.9 ± .48	10.3 ± .44	7.7 ± .64	5.9 ± .27	6.1 ± .34	s-1, -2 for G, -3
		6	4	5	1	2	3	
k	Number, roots > 12 inches	10.0 ± .34	11.0 ± .32	11.3 ± .72	15.1 ± .21	14.9 ± .44	14.4 ± .44	s-1, -3
		6	5	5	3	1	2	
l	Weight of plants grams	423.7 ± 9.79	466.3 ± 13.7	475.0 ± 20.0	549.5 ± 17.6	578.0 ± 16.9	566.8 ± 12.3	s-1, -3
Pineapple yield records								
		6	5	4	2	1	3	
m	Total yield per plot, lbs.	792	1009	1041	1206	1207	1183	79.48 s-1, -2, -3
		6	5	4	3	1	2	
n	Weight per fruit, lbs.	2.74 ± .008	3.35 ± .012	3.38 ± .008	3.91 ± .012	4.03 ± .012	3.94 ± .012	s-1, -2, -3

[a] The figures for critical differences are inserted only for the cases in which complete analyses were made by Fisher's Latin Square method. The significance or nonsignificance of any particular difference is readily determinable in such cases. Other bases of comparson were analyzed by an assistant by the normal curve method, and significances by that method are indicated in the last column as follows: s-1, chloropicrin with checks; s-2, carbon bisulphide with checks; s-3, chloropicrin with carbon bisulphide, C (s meaning unquestionable significance).

eration of plant parts often produced by other types of parasitism. As an immediate effect this is probably beneficial to the plant, but it cannot continue indefinitely, and, in the long run, probably prevents normal new root development when it is later needed. The differences are not great, however, and this basis of measurement, because of the present uncertainty as to influences, can be disregarded in the further consideration of the data.

In all categories of comparison, except that of b, then, the treatments group themselves into 3 classes as to efficiency, always in the same order: first, the 3 chloropicrin treatments, B, D, and F; second, the carbon bisulphide treatments; third, the controls. The differences by which such classes are brought about are, in general, highly significant, statistically. (Note the single partial exception, in category i, and lack of either the chloropicrin-carbon bisulphide, or the carbon bisulphide-check significance in some of the categories of minor influence value.) In the table such grouping is indicated by subdivision into 3 parts by heavy lines. Within the groups, differences are not, as a rule, statistically significant. It is worthy of note, however, that plot G shows superiority over C in all but one of the bases of comparison, indicating that for carbon bisulphide the better confinement of the gas brought about by the additional cover made itself felt in results obtained. Such benefit from the extra cover in the case of chloropicrin treatments is not evident.

Results in Plant-crop Yields.—The ultimate criterion upon which judgment as to the value of any treatment for pest control is based is its effect on crop yield and quality. Measurements on yield for this experiment covered only "plant-crop," that is, the first fruiting, from July to September, inclusive, 1932. The data in total plot yields, analyzed by the Latin Square method, gave the following results.

Standard deviation due to random error, 48.58 pounds; critical difference (as explained heretofore) 79.48 pounds.

Average yields by treatments are appended to table 7. These data show at a glance the significant differences. They include all treatments over the checks and, very definitely, the chloropicrin over the carbon bisulphide. Percentage increases in yields from the treatments were as follow:

C	over	checks,	27.4	per	cent
G	"	"	31.3	"	"
B	"	"	52.2	"	"
D	"	"	52.3	"	"
F	"	"	49.3	"	"
B	over	C,	19.5	"	"

The economic significance of such increases is considered in the "Discussion" section of this paper.

An additional analysis of yield data, based upon individual fruit weights throughout, gives the results in average weight per fruit by plots.

These data are analyzed by the normal-curve method, since the populations were well over 2000 per treatment. The results are included in table 7 as the last category of comparison (n). They show much the same order as do total plot yields, the only differences in order being between chloropicrin treatments with which, in total yields, differences are not significant. Differences in numbers of fruits weighed, not accounted for by methods of record taking, make for the slight discrepancies that occur. Again, marked benefit from the chloropicrin treatments is evident.

Consideration of the Correlations Between the Different Bases of Comparison.—Further critical consideration of the data presented in table 7 shows, for the 3 main classes of results (chloropicrin, carbon bisulphide, and control) practically perfect rank-order correlation throughout, except for basis b, which may be disregarded. An analysis of this situation leads to the following conclusions:

(1) The nematode populations left after the treatments, as measured by basis a, accounts for the orders of degrees of nematode infection in the pineapple plants, as given in bases e, f, g, and h.

(2) These infections, in turn, affect root growth as manifested by bases c, d, j, and k.

(3) The extent of root growth probably is an important factor in plant growth, as shown by basis l.

(4) All such factors influence yield, as shown by m and n.

Thoroughgoing statistical treatment of these various categories of data is not included in the present paper, for they are not essential to its main purpose, *i.e.*, to show the results of the treatments on the pineapple crop. One correlation, however, that between a and m (indicator crop readings and plot yields), is reported as of definite interest in connection with nematode-control work, in general. Calculations were made on individual plot data, the population, therefore, being 49 plots. The step intervals were 10 galls per plant for indicator-crop data and 50 pounds per plot for yields. The result of this calculation was $r_{ma} = -.852 \pm .0265$; a surprisingly high correlation, considering the admittedly rough method of obtaining the data on gall counts. An undoubted influence of nematode population on pineapple yield is manifest, unless other factors, simultaneously influencing yields, were affected by the treatments, and nematode readings served as a measure of the effects on these other factors, as well.

The regression equation based upon this correlation is $M = -39.5\,A + 1242 \pm 34$. By way of example of its application to the conditions of this experiment, an indicator-crop planting of sufficient magnitude (6), grown in midsummer under optimum conditions for growth and for infection, giving a reading of say 100 galls per plant, would promise a yield of $-39.5 \times 10 + 1242 \pm 34$, or 847 ± 34 pounds per plot, or, multiplying by $\frac{43560}{900}$

and dividing by 2000, 20.469 ± 1.122 tons to the acre. Had the gall count averaged only 20, or 1/5 as heavy infestation, the yield indicated would have been 1163 ± 34 pounds per plot, or 28.106 ± 1.122 tons per acre. This represents an increase in yield of better than 37 per cent correlative with the improved condition with regard to nematodes.

Naturally, this equation can not be applied generally in estimating yields, for every locality varies in many other yield-influencing factors besides nematode infestation of the soil. Even in the same locality, others of such factors might vary independently of the nematode population to such an extent as to outweigh the nematode factor. It is to be presumed in the experiment just described that the treatments may have reduced other detrimental influences aside from nematodes, and such reductions may have had their influence on yield, as well. Another type of nematode control not influencing such other factors in like degree might bring nematode infestation down to points equal to those here shown without other beneficial effect, in which case yields would not be influenced so greatly. It goes without saying that it is unsafe to rely upon the results of a single such experiment as this for governing field practice. Nevertheless, since the chemical treatments described were the only variables introduced, all other conditions in all plots being as nearly identical as possible under field conditions, considerable stress can be laid upon the reliability of the results reported.

Results of Simultaneous Single-row Tests with Cyanogas and Potassium Xanthate.—Immediately after the installation of experiment 2 as a Latin Square experiment, there being still space for several more rows of the same heavily infested land, a portion of such space was devoted to tests of Cyanogas and potassium xanthate with a view to determining their relative efficiency at different rates of application. The Cyanogas, in granular form, was applied by hand in furrows cut with a plow down the center and on either side of the plant bed, 3 lines in all. The potassium xanthate was similarly applied but with the addition of an equal quantity of acid phosphate, as supplied by the fertilizer trade, in accordance with the recommendations of deOng and Tyler (3). The soil in this section is decidedly on the acid side of neutrality (pH 4 to 5), and was not dry, as shown by the good growth of indicator plants without irrigation; so conditions should have been favorable for best results with this chemical. In both cases, the furrows were filled by cultivators immediately after application of the chemicals, and mulching paper covers were applied as for regular planting.

Rates of application, together with results of treatments in terms of average weight per fruit harvested, are given in table 8.

Casual observations made from time to time during the growth of the plants disclosed definite superiority in plant growth over near-by nontreated

TABLE 8.—*Pineapple-yield data on single-row treatments with Cyanogas and potassium xanthate*

Row	Treatment	Rate per acre in pounds	Number of fruits harvested	Average weight per fruit
43	Cyanogas	870	155	3.20 ± .040
44	"	580	197	3.11 ± .037
45	"	1164	193	3.13 ± .034
46	Xanthate	500	180	3.15 ± .039
47	"	340	167	2.89 ± .043
48	"	680	181	2.97 ± .040
	Checks	(of experiment 2)		2.74 ± .008

rows and over the controls of experiment 2. The yield results show definitely significant increases in weight per fruit over the controls, except in the case of the lightest xanthate treatment. The increases are not sufficient, however, to justify their consideration in comparison with those shown for chloropicrin in experiment 2. From a superficial examination it would appear at once that the costs of the applications are such that their use would be profitless.

DISCUSSION OF EXPERIMENTAL RESULTS

Since the technique of application of chemicals was definitely better in experiment 2 than in 1, without increase in costs, and the results obtained better and probably more significant, the discussion of the economic considerations is confined to the results from experiment 2. Reexamination of the final results in yields per plot (basis m, table 7) discloses definitely increased yields from the chloropicrin applications over the controls. Taking treatment B, 150 pounds of chloropicrin per acre, as a basis of comparison, the increase is 413.5 pounds per plot. Increasing this by 4.44 per cent (because of the removal of this proportion of the plants for earlier examination) the increase to be expected on the field scale is 431.8 pounds. This amounts to 10.435 tons per acre. A conservative estimate of the field value of fruit, based upon the average through 20 years, is 1 cent per pound. The cost of 150 pounds of chloropicrin at 80 cents per pound is $120.00, and the cost of application about $5.00 per acre. With these figures as a basis, the following calculation can be made:

Value of 20870 pounds increase at 1 cent per pound	$208.70	
Cost of chloropicrin treatment	125.00	
Profit arising from the treatment		$83.70

This profit is in plant crop tonnage alone. As additional credits to the chloropicrin should be added 2 items: (1) The higher grade of the fruit

throughout because of the much larger proportion of first-grade fruits on the basis of weights alone (average weight 3.912 pounds as compared with 2.744 for the checks); and (2) the fact of the stand of plants still being in excellent condition after plant crop and, therefore, capable of producing one or more ratoon crops, whereas the check-plot stands were almost worthless in that respect. It would be difficult to estimate the economic value of these additional credits without actual measurements, but they are certainly worthy of consideration. On the basis of plant-crop yields alone, however, the treatment would appear to be justified when it is necessary to plant fields known to be heavily infested with nematodes. Different estimates for basic costs and values would give different figures for profits, but those used can not be far from the average. The feasibility of such treatments as regular field operations under heavy infestation conditions is a matter that can be determined only by consideration of all the economic factors involved.

Similar treatment of the carbon bisulphide data shows a considerably smaller margin between costs and profit from the increased yield, leaving the advantage decidedly in favor of the chloropicrin treatment. The same applies to the Cyanogas and potassium xanthate treatments.

SUMMARY

Two field experiments on the control of the root-knot nematode *Heterodera marioni* in heavily infested Hawaiian pineapple fields, incorporating the use of chloropicrin and certain other chemicals, were conducted in 1931 and 1932. Both were installed with the Latin Square arrangement of plots in order to eliminate soil heterogeneity in infestation. The chemicals were inserted into well-prepared beds that were immediately covered with the usual tar-impregnated, pineapple mulching paper.

Measurements of the effectiveness of the treatments were made by gall counts on cowpea indicator-plant roots, studies on excavated random selected pineapple plants, and, finally, by yield records. In experiment 1, chloropicrin was injected through holes in the paper cover, which were then plugged with soil. In spite of this inefficient confinement of the gas, the following significant benefits resulted from the treatments:

97 pounds per acre, 14 per cent yield increase.
130 pounds per acre, 94.5 per cent reduction in nematodes, 26 per cent yield increase.
160 pounds per acre, 94.4 per cent reduction in nematodes, 31.4 per cent yield increase.

In experiment 2, gas confinement was more efficient by virtue of unbroken paper-mulch covers, throughout. In this case, a comparison with

carbon bisulphide was incorporated. Significant results, in condensed form, are as follows:

C Cl_3NO_2 150 lbs., nematode reduction 83 per cent, yield increase 52.2 per cent.
C Cl_3NO_2 170 lbs., nematode reduction 90 per cent, yield increase 52.3 per cent.
C S_2 750 lbs., nematode reduction 48 per cent, yield increase 29.3 per cent.

Additional data on effects of the treatments on various features of pineapple plant growth and root gall development are tabulated as well. A correlation of $-.852 \pm .0265$ is shown between the original indicator-plant root-gall count and pineapple yields, for the 49 plots of experiment 2. This would appear to establish a definite and highly important detrimental influence of a high initial nematode population on pineapple yields. Definite superiority of chloropicrin at 150 pounds per acre (as applied in plant beds only) over carbon bisulphide at 750 pounds, is shown.

With chloropicrin at 80 cents per pound and a valuation of 1 cent per pound for pineapples, a substantial profit ($83.70 per acre in plant crop alone) from the use of chloropicrin in badly infested fields is established.

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THE RASPBERRY WHITE-BUD DISEASE AND ITS RELATION TO BITTER ROT OF APPLES[1]

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The occurrence of a disease on red raspberries caused by a Glomerella similar in many respects to the bitter-rot fungus suggested the importance of determining its true identity. This disease was of particular concern because apple bitter rot is seldom found in the vicinity of Urbana, Illinois, or north of the latitude of Springfield of the same State (2). Yet, some raspberry plantings were severely infected within this area, by an apparently related fungus. Because of its potential importance to the apple and raspberry industries, the problem of determining the relationship of the raspberry disease to apple bitter rot was undertaken in this investigation.

NATURE OF THE DISEASE

Name.—Due to the whitish gray color of the infected tissue and the fact that the lesions usually occur around the lateral buds this disease has been given the common name "white bud."

Symptoms.—Raspberry white bud is first evident in midsummer as a dark brown discoloration advancing from the vicinity of the bud on the young canes. A few cases have been observed where exposed surfaces on the petioles, near the base, became infected, but generally the fungus seems unable to infect through the weathered epidermis (Table 4). The lesion gradually turns whitish gray following leaf fall, when numerous acervuli are produced, appearing as little black dots over the surface of the lesions. In a mature lesion the bud is usually surrounded by infected tissue. The infection may proceed up the leaf petiole, turning it whitish gray at the base. The leaf falls prematurely and the lateral bud usually dies. It may be killed by direct invasion of the fungus or indirectly through death of the phloem and cortical tissues of the cane to which the bud is attached. The lesions seem always to be localized at the point of union of the cane and the leaf petiole on the shoots (Fig. 1). No leaf infections or infections of second year wood have been found in this study. The disease is most evident during the dormancy of the infected canes. Schwarze (11) reports an abundance of perithecia on the lesions in the spring. Only a few immature

[1] Contribution from the Department of Horticulture, University of Illinois, Urbana, Illinois. The writer wishes to acknowledge his appreciation to Dr. H. W. Anderson and the late Dr. F. L. Stevens for many helpful suggestions and criticisms in carrying out the experiment and preparing he manuscript; to Dr. A. S. Colby for furnishing raspberry varieties for the experiment.

ones have been found under field conditions in Illinois, but mature ascospores have been produced in the laboratory on bean-pod agar.

Host Relationships.—An opportunity was afforded to study the relation of the disease to varieties of raspberries and apples by the occurrence of the disease in the extensive raspberry variety planting on the university farm. Many apple varieties susceptible to bitter rot (Anderson 1) were directly adjacent to the diseased raspberries. This is of significance, since

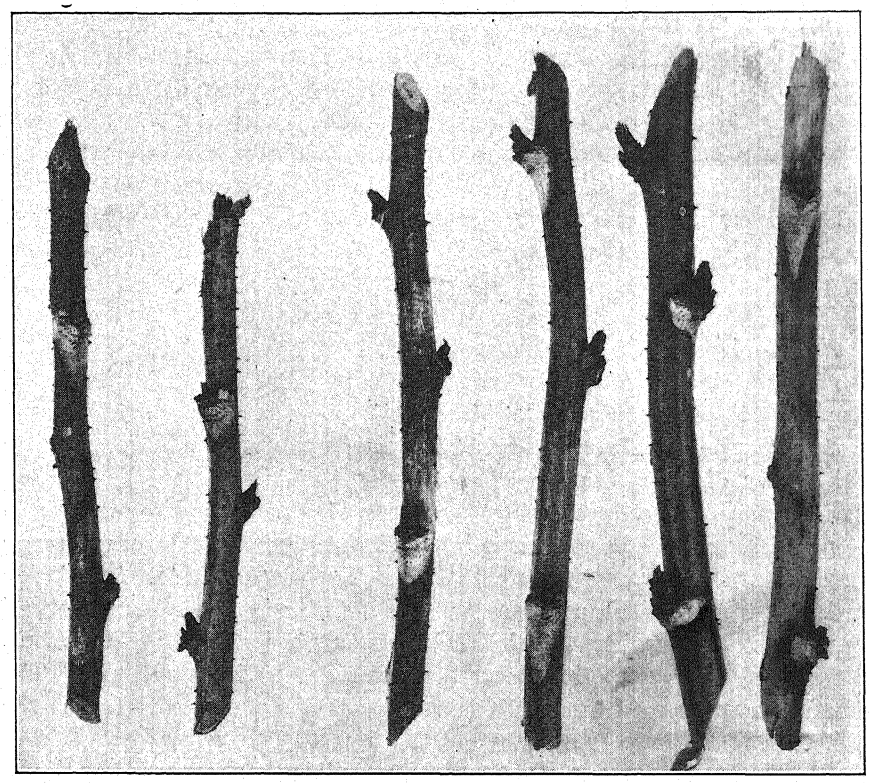


FIG. 1. Raspberry canes, Erskine Park variety, with natural infections by *Glomerella rubicola.*

it will be shown later that if wounds are present to allow its penetration, the white-bud fungus will cause typical bitter rot of apples.

When the fungus is present on the Erskine Park raspberry, nearly all buds are killed. The following varieties usually show between 25 per cent and 50 per cent of their buds killed: June, Perpetuelle de Billiard, Chief, King of Market, Idaho, Newburgh, Jackson, Spineless, and Thornber. Light infections (less than 25 per cent bud kill) may occur on the following varieties: Geneva Station Nos. 2491, 2568, 2584, 1235, Baumforth's Seed-

ling, Victory, Owasco, Rathman, Latham, Fewthorn, and Cayuga. Although 110 varieties of apples were growing immediately adjacent to the diseased raspberries, no bitter rot was found on them.

Economic Importance.—Fortunately the disease is of no great importance on leading commercial varieties, but because of the constant introduction of new raspberry varieties, it may ultimately be of general concern. In this State it has been found only on red raspberries. It appears to have been reported from Canada, New Jersey, and Illinois, but it is undoubtedly present in other States. It could easily be confused macroscopically with raspberry spur blight, *Didymella applanata* (Niessl) Sacc.

Cause of the Disease.—Many typical white-bud lesions from different raspberry varieties were examined in the laboratory. The small black dots, so general on mature lesions, were typically the acervuli of a Colletotrichum. The conidia measured 11–18 μ by 3–7 μ (Fig. 3), and setae were usually present in abundance on infected canes. No mature perithecia were found on the diseased canes, although several immature ones were observed. They are produced on the same sori with the acervuli. The fungus seemed to be identical with *Glomerella rubicola* (Ston.) S. & S.

EXPERIMENTAL COMPARISONS OF APPLE BITTER ROT AND RASPBERRY WHITE BUD

Since a study of the literature of this species indicated some confusion regarding its identity and possible relationship to the bitter-rot fungus, a comparative study of the two organisms was undertaken.

Cultures Used.—The raspberry white-bud fungus was secured by the poured plate method from acervuli from typical lesions. All cultures from different diseased varieties appeared identical. The culture of *Glomerella cingulata* (Ston.) S. & S. used in the following comparisons was obtained from F. L. Stevens, who isolated it recently from diseased apples sent from southern Illinois.

Cultural Studies.—Comparisons of the apple bitter-rot and the white-bud fungi were made on various culture media. The cultural characters are listed in table 1 and shown in figure 2. It may be seen from table 1 that the two fungi differ culturally in several respects, but whether or not these variations are within the limits of variability shown to exist for *Glomerella cingulata* by other workers can not be definitely determined by so limited a comparison. Certainly the differences are sufficient to merit specific rank in groups of more stable fungi. The ascigerous stage was not produced in culture by either fungus when subjected to the ultra-violet ray treatment of Stevens (13), which was found so successful with some strains of the bitter-rot fungus, but it did stimulate acervuli production by the bitter-rot fungus. The aerial growth of both fungi was decreased mark-

edly by this treatment. The perfect stage of the white-bud fungus was produced on bean-pod agar after 6 weeks incubation at room temperature and agrees in general with descriptions of *Glomerella cingulata* as well as the available descriptions of *Glomerella rubicola.*

Ascospore measurements of the raspberry Glomerella show that they fall within the range of the measurements reported for *Glomerella cingulata.* This fact is not especially significant, since the ascospore measurements of the latter species as reported by various investigators vary as much

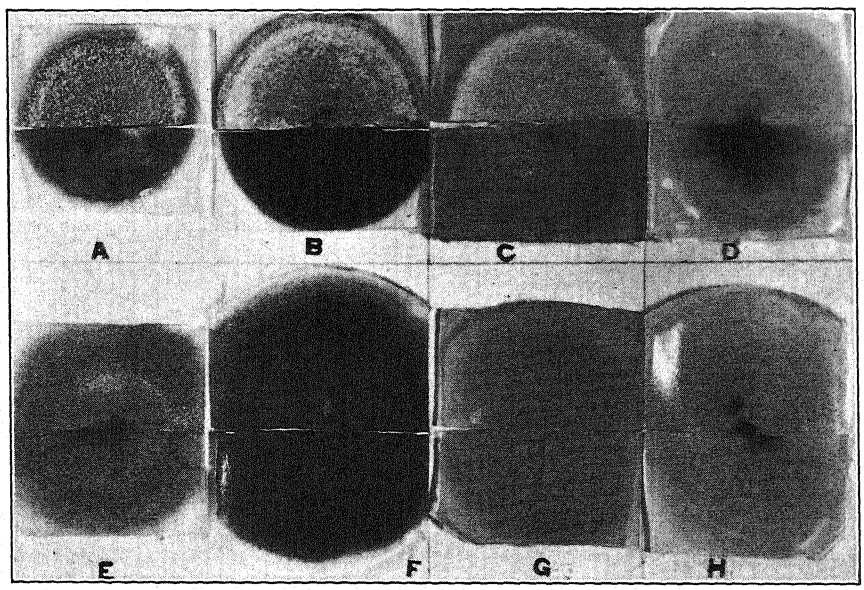


FIG. 2. Top and bottom views of cultures grown for 10 days at room temperature on various agars. A–D. Glomerella from Rubus. E–H. *G. cingulata.* A and E, on prune; B and F, on 2 per cent potato dextrose; C and G, on bean pod; and D and H, on corn meal.

as 16 μ in length and 5 μ in diameter. Spore graphs of the conidia of these two fungi are presented in figure 3. The conidia of the white-bud fungus are slightly longer than those of *Glomerella cingulata.* Again it is difficult to properly evaluate this difference since the literature contains measurements of the conidia of *Glomerella cingulata* much larger than those found by the writer. Another point of interest, but of little morphological value, is the fact that setae are present in abundance in the acervuli of the white-bud fungus as it is found on the infected canes, but have not been observed when this fungus produces acervuli on inoculated apples. Also, a few cases of the production of setae on inoculated apples by the bitter-rot fungus have been observed in this study. That setae are of little value in

TABLE 1.—*Cultural differences of the Glomerella from Rubus and G. cingulata from apple after 10 days' incubation at room temperature*

Media	Color of Cultures (Ridgeway (9))		General Habit	Spore Habit
	Below	Above		
A[a]—Prune *G. rubicola*	Garnet brown (I–k) to Hermosa pink (I–f) Margin white—very variable	Whitish-pink Variable	Fluffy growth above media, 1½ in. growth in 7 days—rudimentary acervuli	Prolific sporulation mostly above the medium
E—*G. cingulata*	Pale olive gray (LI–f) Light around edge	Whitish-gray with blackish streaks	Very slight aerial growth—great abundance of acervuli	"
B—Potato dextrose 2% *G. rubicola*	Hazel (XIV–k) narrow white edge	Pink and fluffy	Fluffy growth with much piling up—2″ in 7 days No acervuli	Prolific sporulation
F—*G. cingulata*	Light buff (XV–f). Along edge—nearly solid black with buff spots	Same—very little aerial growth	Very great acervulus production	"
C—Bean pod *G. rubicola*	Light buff (XV–f)	White and fluffy	No acervuli. Fluffy aerial growth common	Sporulation medium. (Perithecia produced after 6 weeks)
G—*G. cingulata*	Light buff (XV–f)	Light buff to white on margin	No acervuli produced	Sporulation heavy
D—Corn meal *G. rubicola*	Etruscan red (XXVII–b in center to olive buff (XL–d) on margin	Fawn color (XL–b) with spots of Etruscan red	Little tufts of aerial mycelium growth mostly in media. No acervuli	Sporulation medium
H—*G. cingulata*	Pale neutral gray (LIII–d)	Same	Growth mostly in media. Acervuli abundant	"

[a] Letter corresponds to letters on figure 2.

the taxonomy of fungi has been demonstrated by other workers. In brief it may be said that so far as the two fungi under consideration are concerned, they show rather striking cultural differences; but since the general group of anthracnose fungi are known to vary considerably, both culturally and morphologically, the variations encountered may not be sufficient to regard them as distinct. It seems, therefore, that the only hope of reliable separation of these two fungi is in their host relations.

Inoculation Tests.—In table 2 all of the apples were inoculated by Huber's (6) method. The columns marked "Positive results" indicate the

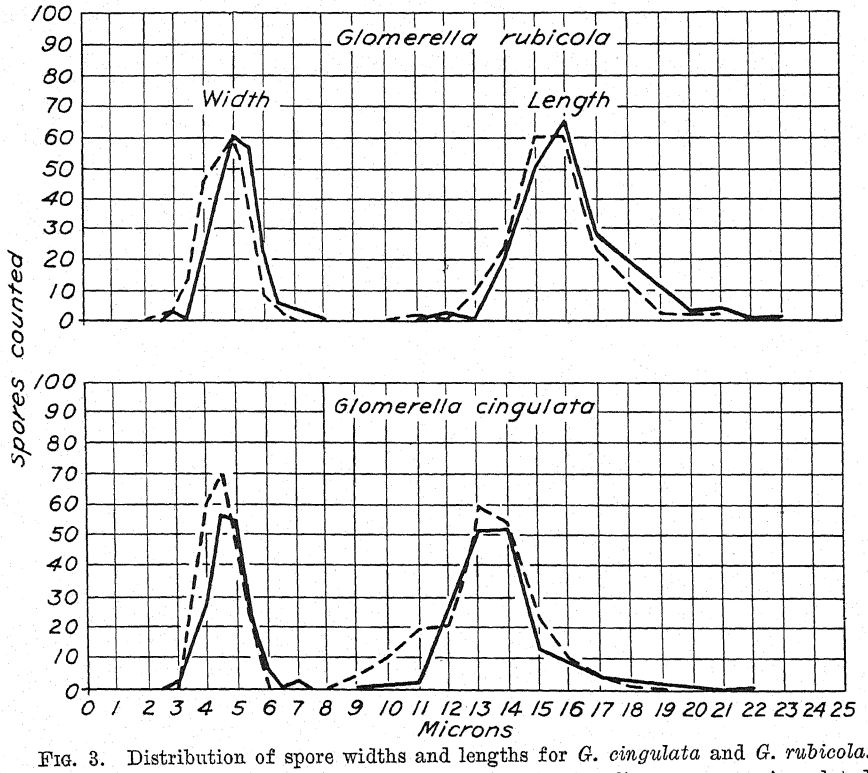


FIG. 3. Distribution of spore widths and lengths for *G. cingulata* and *G. rubicola*. Solid line, grown on 2 per cent potato-dextrose agar. Broken line, grown on inoculated apple.

number of successful inoculations. No contamination was in evidence. The methods of this experiment were fundamentally the same as those followed by Dodge (4), when he successfully produced lesions on raspberry canes inoculated with a strain of *Glomerella cingulata*. He also produced typical apple bitter rot on apples inoculated with the fungus isolated from diseased canes. The results of cross-inoculation experiments of this investigation are listed in table 2. Ben Davis and Grimes apples and the Erskine Park raspberry were used in this experiment.

TABLE 2.—*Cross-inoculations with raspberry white-bud and apple bitter-rot fungi. Inoculated apples held at room temperature. Raspberries were inoculated under greenhouse conditions. Canes inoculated by placing bits of the fungus growth from culture media into wounds made with a scalpel*

Fungus used	Apples			Raspberries	
	Varieties used	Number inoculated	Positive results	No. of canes inoculated	Positive results
White bud	Ben Davis	30	30	20	20
White bud	Grimes	30	30	20	19
Bitter rot	Ben Davis	30	30	20	0
Bitter rot	Grimes	30	30	20	0
Checks	Ben Davis	5	0	5 wounds	0
	Grimes	5	0		

Both fungi caused typical bitter rot of apple when inoculated into the flesh of the apple (Table 2, Fig. 4, A and C). *Glomerella cingulata,* the bitter-rot fungus, did not cause lesions of any type on inoculated canes of the Erskine Park raspberry while the white-bud fungus did in every case but one (Fig. 5). The environmental conditions were the same for both fungi throughout the experiment. These results do not confirm the work of Dodge (4), but it is possible that he was using a different strain of apple bitter rot. The variety of Rubus used might also account for the difference in results. Shear and Wood (12) have regarded the ability of a fungus to produce typical bitter rot of apple when inoculated into the flesh as sufficient evidence for placing several species of Glomerella in the single species *Glomerella cingulata.* There is a question as to whether positive wound inoculations into mature apples constitutes experimental evidence of sufficient value for regarding the fungus as naturally parasitic on apple. Since the Glomerella from Rubus and the Glomerella from apple both caused typical bitter rot of apple, they might at least be regarded as strains of *Glomerella cingulata.* It is evident, however, that the mature apple contains an abundance of readily available sugars, moisture, and other chemicals which are necessary for the growth requirements of a given group of fungi. *Glomerella cingulata* causes great injury to apple crops because the fungus is able to penetrate the unbroken epidermis of the fruit. The fact that one fungus will enter an apple through an unbroken skin and another will not undoubtedly offers a more reliable basis of possible separation than the information gained when the fungi to be tested cause rotting after inoculation by wounding the apple. Evidence of this nature, if used by itself, might indicate nothing more than strains of a given fungus, but if supplemented by other recognized procedures might play a more important diagnostic rôle.

In order to determine whether the fungi under consideration could penetrate the unbroken epidermis of mature apples and cause typical apple bitter rot, the following experiment was carried out. Spores of the Glomerella from Rubus and from *Glomerella cingulata* were atomized onto apples

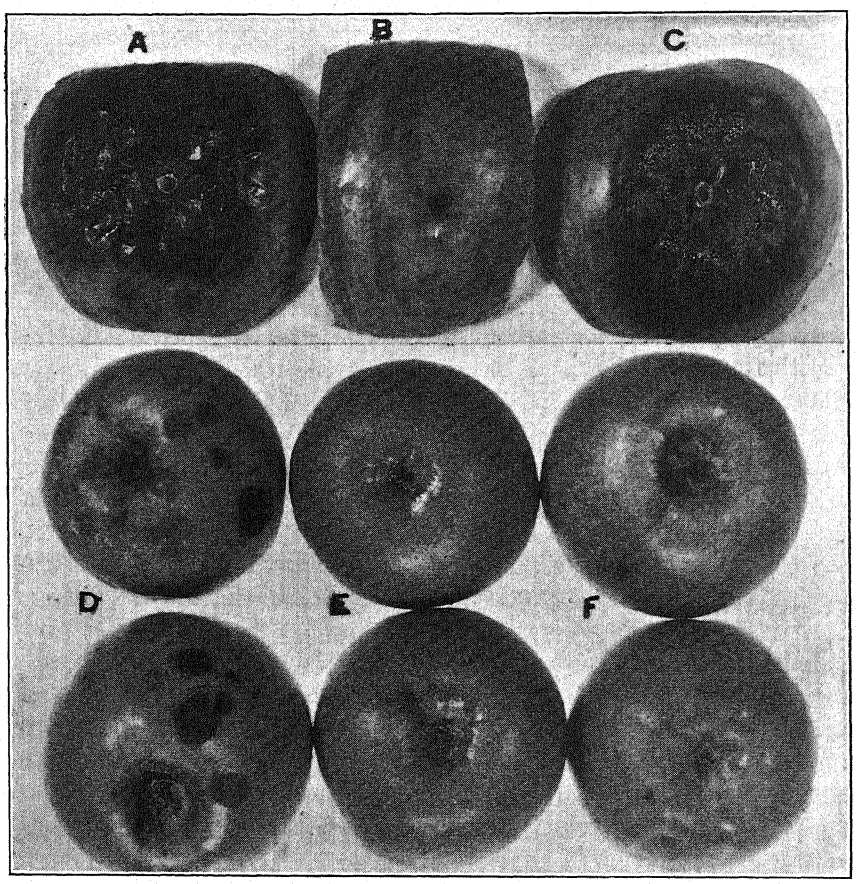


FIG. 4. A to C. Ben Davis apples. A and C. Typical bitter rot produced by artificial inoculations into the pulp with *Glomerella cingulata* and Glomerella from Rubus, respectively. B. Check. D to F. Grimes Golden apples after atomizing the uninjured fruit and keeping two weeks in a damp chamber at room temperature. D. With spores of *G. cingulata*. E. With sterile distilled water. F. With spores of Glomerella from Rubus.

that had been previously sterilized with 1–1000 $HgCl_2$ and rinsed in sterile distilled water. The apples were then placed in damp chambers and held at room temperature for ten days. The results of this test are given in table 3 and illustrated in figure 4, D to F.

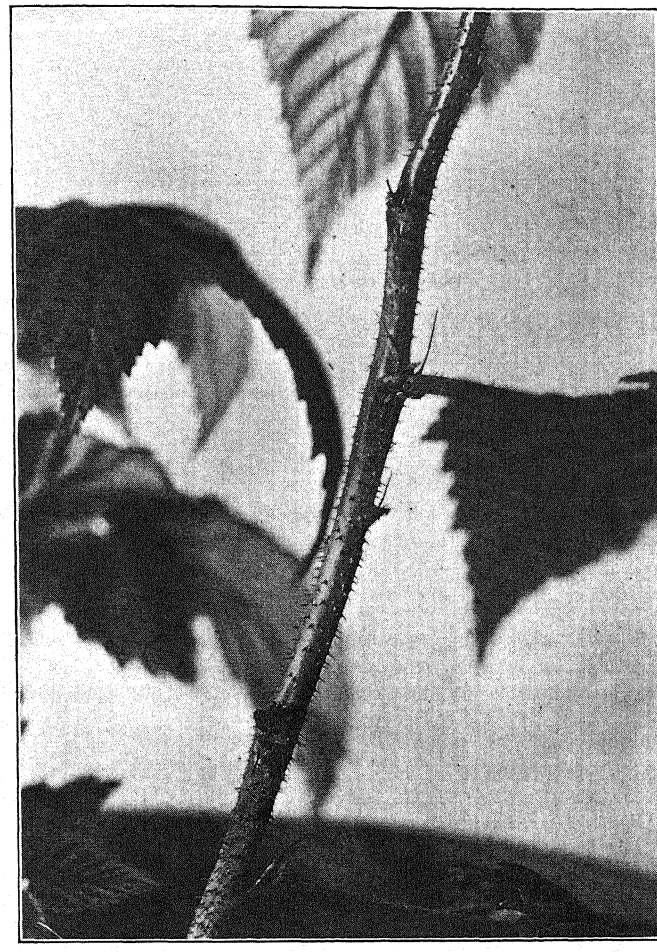


FIG. 5. Young Erskine Park shoot showing lesions caused by Glomerella from Rubus 4 weeks from time canes were inoculated by needle puncture.

The results of this experiment show conclusively that *G. cingulata* will penetrate the unbroken skin of Grimes apples and that the Glomerella from Rubus will not, under the environmental conditions of this test.

A test similar to the one just described was run to determine if the fungi would penetrate the unbroken epidermis of young vigorous raspberry shoots, and, if so, whether entrance was always through the unweathered tissue at the junction of the leaf petiole and the cane, as is evidently the case under field conditions. Table 4 gives the results of these tests.

Apparently the tissue in the vicinity of the lateral bud is the only place where infections take place, unless wounds are present on the canes. *Glom-*

TABLE 3.—*Surface of Grimes apples atomized with spore suspensions of Glomerella from Rubus and Glomerella cingulata to test ability of fungi in question to enter unbroken epidermis of apples. Incubated in damp chambers at room temperature ten days*

No. of apples atomized	Spore suspension	No. of positive infections[a]	Location of lesions
10	*G. cingulata*	93	General
10	G. from Rubus	1	Calyx end
10	Sterile water	0	Centered at worm puncture

[a] Reisolations made from typical lesions and compared with cultures used to make spore suspensions.

erella cingulata did not cause any disease on Erskine Park. The experiments show that the Glomerella from Rubus is capable of causing the white-bud disease by infections through the uninjured epidermis of raspberry canes.

TABLE 4.—*Atomizing spore suspensions of G. cingulata and Glomerella from Rubus on vigorous young shoots of Erskine Park raspberries. Plants held in inoculation chambers for 48 hours after being atomized and then grown under greenhouse conditions*

No. of canes	Spore suspension	No. of positive infections[a]	Location of lesions
5	Glomerella from Rubus	18 buds out of 41	At junction of petiole and cane—one petiole infection
5	*G. cingulata*	None	—
2	Sterile water	None	—

[a] Fungus reisolated from lesions in a few typical cases.

DISCUSSION

Taxonomic Status of Raspberry White-bud Fungus

A Gloeosporium was collected from the leaves of *Rubus strigosus* by Nuttall in West Virginia (1895). It was distributed by Ellis and Everhart as No. 3468 of North American Fungi under the name *Gloeosporium rubicolum*. Later it was described under this name by Millspaugh and Nuttall (7, p. 123) who credited it to Ellis and Everhart. Specimens of apparently the same collection were sent to Miss Stoneman (14, p. 108–110) who had evidently overlooked both the exsciccati specimen and the previous de-

scription by Millspaugh and Nuttall (7, p. 123), since she changed the name to *Colletotrichum rubicolum* E. & E. Because setae were evidently present in the material she examined, it is possible that she purposely changed the generic name. Later, Stoneman (14) found the perfect stage in culture and described it as *Gnomoniopsis rubicola,* which was changed by Von Schrenk and Spaulding (9, 10) in 1903 to *Glomerella rubicolum* (E. and E.) S. and S. Another Gloeosporium was described by Ellis and Everhart from *Rubus villosus* as *Gloeosporium rubi.* Since this fungus occurs on blackberries and since the spores are much narrower than those of *Glomerella rubicola,* it is evidently a different species. Shear and Wood (12) regard *Gloeosporium rubi, Glomerella rubicola* and *Glomerella cingulata* as synonomous. They imply that Stoneman (14, p. 108–110) regarded the imperfect stage as *Gloeosporium rubi,* but, according to the article they cite, she reports the imperfect form as *Colletotrichum rubicolum.* Both Stoneman (14) and Atkinson (Stoneman (14) Page 81) regard *Gloeosporium rubi* as distinct from *Glomerella rubicola* (*Colletotrichum rubicolum*). In 1914 Schwarze (11) described and figured a disease on the stems of raspberries, which was ascribed to *Glomerella rubicola* (Ston.) S. and S. and listed as the imperfect stage as *Colletotrichum rubicolum.* Since this fungus was found on canes while the original species occurred on the leaves, it is presumed that he based his identification on morphological details. Judging from his account of the disease it is identical with the one studied in these tests. In 1908 Edgerton (5) successfully rotted apples with a Colletotrichum taken from raspberry stems. From available descriptions it is impossible to determine whether or not Edgerton regards the form on stems as being identical with the form described on leaves by Stoneman (14). Shear and Wood (12) regarded the experiment of Edgerton as sufficient evidence to consider the apple bitter-rot fungus and the raspberry Colletotrichum as identical. Dearness (3, p. 68) lists *Glomerella rubicola* as being present in Canada, but does not give a description of the disease caused by it. In 1927 Dodge (4) published the first work with sufficient experimental evidence to justify the conclusion that the form he described on the raspberry is the same or very closely related to the apple bitter-rot fungus. His descriptions and figures, as well as the experimental evidence presented lead to the conclusion that the form worked with is not the same as the one under consideration. Other Gloeosporia have been described on *Rubus* spp., but are not included in this discussion because their morphological characters eliminate them as possible synonyms.

CONCLUSION

From the foregoing investigation it is believed that the white-bud fungus is a distinct species and should not be regarded as a strain of the bitter-rot

fungus. While according to Dodge (4) strains of *Glomerella cingulata* are capable of infecting raspberries, the symptoms produced by this fungus are not typical of those produced by the white-bud fungus. *Glomerella cingulata* from apple bitter rot infects sound apples through the unbroken epidermis, while the white-bud fungus does not; consequently the pathogenic properties of the two are distinct. There are also variations in cultural and morphological characters that are in themselves of less importance in this variable group, but when taken in conjunction with the decided difference in pathogenic properties may be regarded as significant. Since the name, *Glomerella rubicola,* has been given to a fungus producing similar symptoms on raspberries to those herein described and since the morphological characters agree, it is thought best to retain this name for the white-bud fungus.

SUMMARY

1. Raspberry white bud is a disease found only on red raspberries in Illinois. On certain varieties it is very injurious.

2. The white-bud fungus is distinct in pathogenicity, symptoms, and in cultural and morphological characters from the strain of *Glomerella cingulata* generally present in Illinois.

3. The white-bud fungus will cause typical bitter rot of apples if inoculated into them.

4. In inoculation tests on Erskine Park the white-bud fungus gave positive results, while the strain of *G. cingulata* used did not.

5. *Glomerella cingulata* spores atomized on apples infected them through the unbroken epidermis. The Glomerella from Rubus did not infect apples under the same conditions.

6. The Glomerella from Rubus caused the white-bud disease of Erskine Park by direct penetration of the epidermis in the vicinity of the buds, but *G. cingulata* under similar conditions did not.

7. Evidence is presented to justify the conclusion that the white-bud disease is caused by *Glomerella rubicola* (Ston.) S. & S.

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THE QUALITY OF LINT AND SEED FROM COTTON PLANTS WITH PHYMATOTRICHUM ROOT ROT[1]

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In a separate paper,[2] the writers have discussed the yield secured from cotton plants known to have succumbed to root rot, caused by *Phymatotrichum omnivorum* (Shear) Duggar, at various periods during the season. The present paper is concerned with the effect of the disease on the *quality* of the lint and seed picked from these same plants.

MATERIAL AND METHODS

The cotton lint and seed were picked in 1931 from Startex (Texas Station No. 7000) plants, growing in an experimental field at College Station, Texas. At weekly intervals during the season, plants killed by root rot or wilted from advanced injury by the disease were marked with small stakes bearing letters to indicate the dates when the plants had apparently succumbed. On September 10 and 28, the cotton from each plant was picked into a separate envelope, dried in the laboratory for one month, and ginned and weighed separately for each plant. Summaries of the yields obtained have been given in the previously mentioned paper.[2] The results for the individual plants have been considered together in lots consisting of plants that succumbed to root rot within approximately weekly intervals during the season.

The length of lint was determined prior to ginning. Where possible, 25 individual seeds were selected for each lot, each seed being taken from the central seeds of a lock from a different plant. The 25 plants selected for this purpose constituted "stratified" samples and were selected to include for each lot proportionate numbers of the plants bearing 1, 2, 3, and so on bolls per plant, rather than merely random samples from the mixed crop. In the lots that succumbed to root rot early in the season there were less than 25 plants that bore cotton, and several seeds were, therefore, selected from each plant of these lots. The lint was measured in millimeters, as summarized in table 1.

Counts were made of seeds from 10 representative plants of those lots in which 10 or more plants bore cotton. These counts, together with the data on yields of these plants, furnished the basis for caculation of lint indexes, as given in table 2.

[1] Published with the approval of the Director as Contribution No. 295, Technical Series, Texas Agricultural Experiment Station.

[2] Ezekiel, Walter N., and J. J. Taubenhaus. Cotton crop losses from Phymatotrichum root rot. (In press). Jour. Agr. Res. 49: 1934.

TABLE 1.—*Lengths of cotton lint produced by plants that succumbed to Phymatotrichum root rot at different periods during the summer of 1931. (Determined from 25 individual measurements of combings of lint from different plants, except only 10 measurements made for lot 2)*

Lot No.	Interval during which plants succumbed to root rot	No. of plants from which lint was measured	Range of individual measurements	Coefficient of variability of measurements[c]	Mean length and probable error
			mm.	*mm.*	*mm.*
1[a]	to June 30	5	12–25	17.2	21.84 ± .52
2[b]	July 1 to July 7	1	10–20	26.0	15.00 ± .88
3	" 8 to " 15	6	9–26	24.8	21.04 ± .72
4	" 16 to " 22	13	17–28	14.6	23.20 ± .47
5	" 23 to " 30	22	12–33	20.9	23.52 ± .68
6	" 31 to August 4	25	17–29	14.1	24.24 ± .47
7	August 5 to August 11	25	20–28	10.2	24.24 ± .34
8	" 12 to " 18	25	17–28	11.2	23.76 ± .37
9	" 19 to " 25	25	22–27	6.0	24.44 ± .20
10	" 26 to September 3	25	19–27	8.4	22.88 ± .26
11	September 4 to September 10	25	17–28	10.6	23.84 ± .35
12	Normal plants	25	15–30	13.2	23.80 ± .43

[a] Two of the 8 bolls borne by plants of this lot were on plants that recovered and were still green on September 10.

[b] The entire crop of this lot was one boll borne by a plant that recovered and was green on September 10.

[c] The coefficient of variability = 100 times the standard deviation, divided by the mean.

TABLE 2.—*Lint indexes of cotton produced by plants that succumbed to root rot at different periods during the summer of 1931. (Mean values in this table refer not to all plants of various lots, but only to the 10 or fewer plants used in calculation of indexes)*

Lot No.	Interval during which plants succumbed to root rot	No. of plants for which indexes calculated	Mean number of seeds per plant	Mean weight per seed, gm.	Mean percentage lint	Lint indexes	
						Range of indexes of individual plants	Mean per lot
1[a]	to June 30	5	38.8	0.07	42.4	1.5–. 6.8	4.6
2[b]	July 1 to July 7	1	27.0	0.08	42.1	5.9	5.9
3	" 8 to " 15	6	46.6	0.04	48.7	1.3– 3.5	2.4
4	" 16 to " 22	10	41.4	0.03	42.5	1.3– 2.7	2.0
5	" 23 to " 30	10	83.9	0.04	40.1	1.5– 4.6	2.7
6	" 31 to August 4	10	62.5	0.06	41.0	2.4– 5.6	3.7
7	August 5 to August 11	10	80.2	0.07	36.8	1.7– 8.4	4.0
8	" 12 to " 18	10	72.4	0.07	40.1	2.6– 8.2	4.7
9	" 19 to " 25	10	81.4	0.10	40.5	4.8– 8.8	6.7
10	" 26 to September 3	10	74.4	0.12	39.8	5.2–10.5	7.9
11	September 4 to September 10	10	64.4	0.11	40.1	4.1– 8.5	7.3
12	Normal plants	10	87.0	0.10	41.0	4.5– 8.9	6.9

[a] Two of the 8 bolls borne by plants of this lot were on plants that recovered and were still green on September 10.

[b] The entire crop of this lot was one boll borne by a plant that recovered and was green on September 10.

After ginning, the lint and seed from the individual plants were massed together into the lots representing the total crop produced by plants succumbing to root rot at the various periods during the season. The combined lint samples were given commercial classing by J. G. Powers (Table 3) of the Department of Textile Engineering of the Agricultural and Mechanical College of Texas. Microscopic study of these same cotton fibers was made later and is presented as the third paper of this series.[3]

The cotton seed from the various lots was divided into 2 portions. One portion was used for germination tests, and another was analyzed by the Division of Chemistry, Texas Agricultural Experiment Station. The results of these analyses are given in table 4.

RESULTS

Length of Lint

The measurements summarized in table 1 indicate a slight but definite effect of root rot on the length of lint. The lint from the 5 lots of plants that succumbed prior to July 31 averaged only 20.9 mm., as compared to 23.9 mm. average for the other 6 lots and 23.8 mm. for lint from the normal plants. Omitting the scanty data for lot 2, the mean lengths for the other 4 lots succumbing prior to July 31 averaged 22.40 mm. $\pm$ .30 mm., as compared to 23.90 mm. $\pm$ .14 mm. for the other 6 lots of root-rot plants. The difference between these means, 1.50 $\pm$ .33, while slight, amounted to about $4\frac{1}{2}$ times the probable error and was thus statistically significant. A possible indirect effect of root rot on the length of the ginned fiber will be discussed below.

Lint Index

A measure of the abundance of lint on the seeds borne by these plants is furnished in table 2, in the form of the "lint index" for 10 representative plants of each lot. The lint index is the weight in grams of fiber produced by 100 seeds. It was calculated from the data for individual plants by multiplying the weight of 100 seeds by the percentage of lint and dividing by the percentage of seeds. Beginning with very low mean indexes of 2.4 and 2.0 in lots 3 and 4, the indexes increase progressively to 7.9, 7.3, and 6.9 in lots 10, 11, and 12. Lots 1 and 2 are apparently aberrant, with indexes respectively of 4.6 and 5.9; but much of the yield in these lots was on plants that recovered and were still green when the first picking was made. Early attack of root rot, therefore, not only reduces the yield of lint and seed per plant and per boll, as reported in the previous paper, but also reduces the yield per seed.

[3] Stroman, G. N., J. J. Taubenhaus, and Walter N. Ezekiel. Some effects of Phymatotrichum root rot on the microscopic characters of cotton fibers. Phytopath. 25: 126–130. 1935.

TABLE 3.—*Commercial grades of cotton lint produced by plants that succumbed to root rot at different periods during the summer of 1931*

Lot No.	Interval during which plants succumbed to root rot	Grade of staple	Length of staple	Character of staple	Discount from price or premium[c]
			inches		
1[a]	to June 30	Strict Low Middling	31/32	Weak and irregular	65 points off
2[b]	July 1 to July 7	"	3/4	"	175 " "
3	" 8 to " 15	"	15/16	"	75 " "
4	" 16 to " 22	"	13/16	"	125 " "
5	" 23 to " 30	"	31/32	"	65 " "
6	" 31 to August 4	"	15/16	"	75 " "
7	August 5 to August 11	"	1	Slightly irregular	5 " "
8	" 12 to " 18	"	1	Normal	Even
9	" 19 to " 25	"	1+	Weak	5 points off
10	" 26 to September 3	Middling	15/16	Slightly irregular	10 " "
11	September 4 to September 10	"	15/16	"	10 " "
12	Normal plants	"	1	Good character	25 " on

[a] Two of the 8 bolls borne by plants of this lot were on plants that recovered and were still green on September 10.

[b] The entire crop of this lot was one boll borne by a plant that recovered and was green on September 10.

[c] Stated as points, or hundredths of a cent per pound; 100 points is considered as $5.00 per bale difference in price. By "on" is meant that the stated number of points is to be added to the price of middling 15/16 inch, normal character cotton; and by "off" that the stated number of points is to be subtracted from the basis price of middling.

Root rot apparently had little effect on the percentage of lint borne by plants of the various lots (Table 2). There were slightly higher percentages of lint on plants succumbing to root rot early in the season than on those succumbing later.

Commercial Grade of the Lint

The effect of root rot as possibly influencing the price paid for cotton from root-rot plants was determined by actual commercial grading of the massed samples of ginned cotton (Table 3). Lint from plants that succumbed to root rot up to 2 weeks before the date of picking was graded as Strict Low Middling, while lint from plants that succumbed very late in the season and from the normal plants was designated as Middling. In addition, the staple from most of the early lots was characterized as weak and irregular, while staple from the normal lots and from plants succumbing later in the season was of better character.

The length of staple of the various lots of this ginned cotton (Table 3) did not agree exactly with the earlier measurements of length of lint on the seeds (Table 1). This was to be expected, both on account of the smaller number of measurements necessarily made in the commercial grading, and because ginning may have shortened the fibers of the cotton from root-rot plants to a greater extent than the fibers from the normal plants. In the commercial rating, the 6 lots that succumbed to root rot prior to August 5 were all classed as less than 1 inch long, while 4 of the 6 other lots were classed as 1 inch long.

The premium or discount from the selling price for basis cotton was estimated by the classer in the customary way, largely from the grade and the staple length of these various lots as he had determined them. Differences between the various lots were considerable. The 6 lots from plants succumbing prior to August 5 drew discounts of 65 to 175 points, while cotton from plants succumbing later in the season was given only negligible discounts and the normal cotton was assigned a premium of 25 points. In terms of dollars per bale of cotton, these differences indicate a reduction from the average price of around $30 a bale, received by growers for the 1931 crop, to about $21.25 for cotton of the poorest lot. The cotton from the normal plants should have commanded a price of about $31.25 per bale. The average discount for lots 1 to 6, inclusive, was 97 points, representing a price of about $25 a bale for cotton picked exclusively from plants succumbing to root rot early in the season.

In farm practice, however, the yield from root-rot and normal cotton plants is mixed in picking and ginning. From the portions of the total crop borne by plants of the different lots, it was possible to estimate the theoretical effect of the discounts or premiums assigned to lint of the vari-

ous lots, on the price of a mixture of cotton of all the lots. Thus while the staple of lots 1 to 6, inclusive, was heavily discounted, these lots constituted only 5.6 per cent of the total crop, and would presumably have lowered the grade of the entire crop by only about 21.4 cents per bale. For the entire crop, there would have been a net loss of about $1.15 a bale from the price assigned the cotton from the normal plants. It is obvious that this estimated reduction in the quality of this cotton is not necessarily the same as might have been assigned in actual commercial classing of the cotton picked from the field. The presence in the sample of around 6 per cent of inferior cotton would be considered of varied significance by different cotton classers.

Considering only the cotton harvested from plants with root rot, it may be noted that only about 10 per cent of this total was made up of the inferior staple of lots 1 to 6. However, this cotton was picked from a field, under artificial irrigation, in which root rot had continued to spread at a fairly uniform rate during the entire season. Under dry-farming conditions, on the other hand, the disease may kill many plants in infested fields fairly early in the season, only to be checked soon by dry summer weather. The cotton picked from root-rot plants in such fields would, therefore, consist more largely of cotton from plants attacked early in the season. The lint from the root-rot plants would correspondingly cause a greater average reduction in quality of the mixed crop than would be expected from the data from the irrigated plants as given in the present paper. We may suggest that, for dry-farming conditions, from 10 to 20 per cent of the cotton from root-rot plants might be borne on plants that succumbed to root rot early in the season and, therefore, produced staple of inferior quality. For example, if as much as 80 per cent of the total yield was picked from plants that had succumbed to root rot, only 8 to 16 per cent of the total yield would be expected to be of poor quality staple.

Effect of Root Rot on the Quality of Cotton Seed

Germination test of seeds from 9 of these lots showed a general unfavorable effect from early attack of root rot (Table 4). The 3 lowest percentages of germination were obtained in the seed from plants that succumbed to root rot prior to August 5. Seed from plants that succumbed to root rot after this date germinated nearly, if not quite, as well as those from the normal plants.

Chemical analysis of the dried seeds showed a slight reduction in protein content in seeds from the plants that succumbed to root rot early in the season. Lots 3 to 7, inclusive, all contained less than 24 per cent of protein, while lots 8 to 12 all contained more than 24 per cent. The apparently aberrant results in lots 1 and 2 can be explained, as in other relations, by the fact that much of the yield in these lots was on plants that recovered from the disease.

TABLE 4.—*Results of germination tests and chemical analyses of cotton seed from plants that succumbed to Phymatotrichum root rot at various periods during the summer of 1931*

Lot No.	Interval during which plants succumbed to root rot	Germination of seeds	Chemical composition of dried seeds, percentages of		
			Water	Protein	Fat
		per cent			
1[a]	to June 30	[c]	2.04	23.00	11.44
2[b]	July 1 to July 7	[c]	2.64	24.35	13.48
3	" 8 to " 15	34.0	1.50	22.46	6.86
4	" 16 to " 22	82.4	1.30	21.80	5.88
5	" 23 to " 30	47.0	1.91	20.54	6.22
6	" 31 to August 4	28.5	1.61	23.52	8.28
7	August 5 to August 11	62.5	1.49	23.34	11.34
8	" 12 to " 18	67.3	1.80	24.37	12.62
9	" 19 to " 25	73.3	1.72	24.71	14.38
10	" 26 to September 3	76.5	1.30	24.86	18.10
11	September 4 to September 10	57.0	1.35	24.58	17.22
12	Normal plants	67.0	1.50	24.67	18.20

[a] Two of the 8 bolls borne by plants of this lot were on plants that recovered and were still green on September 10.
[b] The entire crop of this lot was one boll borne by a plant that recovered and was green on September 10.
[c] Insufficient seed for germination test.

The most striking effect of root rot was on the fat content of the seeds. Seeds of lots 3, 4, and 5 contained only about 6 per cent of fat. With later attack of root rot, the fat content increased progressively to 18 per cent for seeds from the normal plants. The results with lots 1 and 2 were again out of line. It is to be noted that the fat content of the seeds of lots 7, 8, and 9, from plants that succumbed to root rot only 2 to 5 weeks preceding harvest, were lower than with the normal seed, although not so low as with seed from plants attacked earlier in the season.

Weighting the percentage of fat content in seeds of the different lots by the weight of seeds produced on these plants, an average figure of 14.9 per cent was obtained for all the seed from root-rot plants. This is 3.3 per cent lower than the 18.2 per cent fat content of the normal plants. Most of this loss was confined to the seed from plants that succumbed to root rot prior to August 26. The fat content for lots 1 to 9, inclusive, averaged only 11.3 per cent, while in the 2 lots succumbing between August 26 and September 10, the fat content was 17.8 per cent, not greatly different from that of the normal plants.

The average loss of 3.3 per cent fat content, which was a little more than 18 per cent. of the fat content of the seeds from normal plants, applied to the seed from the particular root-rot cotton plants grown in the experimental field, which was irrigated. As was pointed out above, under dry-farming conditions higher percentages of the plants with root rot may succumb during the early part of the season, when reduction in the quality is greater, rather than later in the season. For dry-farming conditions, the seeds from root-rot plants might have the fat content reduced by as much as 18 to perhaps 35 per cent of the percentage of fat in seeds from normal plants.

The price paid to growers for cotton seed is in some places adjusted for the protein and fat content of the seeds. In such areas there would be direct cash losses to growers from the reduced quality of seeds harvested from plants with root rot. In other areas, where the analysis is ignored in setting prices for individual purchases, the lowered value of seeds from root-rot plants would still necessarily affect the average price paid, even if no particular penalty were assigned to cotton seed from a root-rot field.

SUMMARY

Cotton lint picked from plants in an experimental field at College Station, that succumbed to root rot 5 weeks or more prior to the date of picking averaged 21.47 mm. in length as compared to 23.8 mm. for lint from plants that succumbed later and from normal plants.

The average lint index of cotton from plants succumbing early in the season was markedly lower than with cotton of the other lots, indicating

that root rot reduces the weight of lint borne per seed, as well as per plant and per boll.

Lint from plants that succumbed to root rot 3 weeks or more prior to the date of picking was graded one grade lower than cotton from plants that survived until later in the season or cotton from the normal plants.

Despite the definitely lower quality of lint from the plants succumbing to root rot early in the season, there was a relatively small percentage of inferior cotton in the total crop, due to the meager crop borne by the plants that were attacked early. It is suggested that under dry-farming conditions probably not more than 10 to 20 per cent of the lint from plants with root rot would be from plants that succumbed to the disease early in the season and produced staple of inferior quality.

Cotton seed from plants that succumbed to root rot early in the season were lower in germination, slightly lower in protein content, and considerably lower in fat content, than was the case with seed from plants that survived until later in the summer or remained normal until harvest.

It may be concluded that root rot has a considerable effect on the quality of lint and seed from cotton plants. This effect is directly proportional to the earliness of attack. Plants attacked early in the season bear relatively small crops, thus the portion of the total crop that is of low quality will probably rarely exceed 20 per cent of the yield from the plants that are attacked by the disease. The loss to the individual grower from reduction in the quality of the crop, therefore, is much less important than the reduction in the size of the crop that may result from attack of root rot.

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BACTERIAL LEAF SPOT OF CARNATIONS

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(Accepted for publication May 12, 1934)

The bacterial leaf spot of carnations, *Dianthus caryophyllus* L., caused by *Phytomonas woodsii* is a disease that has been known for many years. Its early history, however, is somewhat confused with several other troubles of this plant. In 1889, Arthur (1) ascribed a spot disease of carnations to bacteria, and subsequently Arthur and Bolley (2) presented a more detailed account of the disease and named the pathogen *Bacterium dianthi,* n. sp. Later Woods (8 & 9), in a series of papers attempted to show that the disease described by Arthur and Bolley was due to insect injuries (stigmonose), that the bacteria were secondary and that *Bact. dianthi,* as described, was not pathogenic. Nevertheless in 1903 Woods (10) did describe a bacterial leaf spot of carnations, but he considered it entirely different from anything hitherto reported. He did not name the causal organism, however, nor did he give an adequate description of it. Smith (7), in a footnote in volume 2 of Bacteria in Relation to Plant Diseases, finally presented a brief and incomplete description of the pathogen and named it *Bact. woodsii,* n. sp. Such is the early history of the disease.

A careful study of the work of Arthur and Bolley leaves one uncertain as to the carnation trouble that they had under observation. Nevertheless, certain of the symptoms reported by them are characteristic of the disease now known to be due to *Phytomonas woodsii.* These workers, furthermore, reported successful inoculation experiments on several species in the genus Dianthus, so they must have been working with a pathogen. *Bacterium dianthi,* the species described by them, was a yellow-pigment producer, and the causal organism of the carnation leaf spot is known to be white. It is, therefore, possible that Arthur and Bolley were working with a mixed culture, a fast-growing yellow saprophyte that obscured the slow-growing *Phyt. woodsii.*

No serious investigation of the bacterial leaf spot of carnations has been undertaken since the above mentioned articles, and no adequate description of the pathogen has been published. It would appear, therefore, from this neglect of the disease that it has been of minor importance. This supposition is not altogether true, however. In certain parts of the country, particularly on Long Island and in the east-central States, the bacterial leaf spot results in serious losses under both field and greenhouse conditions. The disease in the field is very severe during summers of abundant rainfall. It is also troublesome in greenhouses in the early fall when the

weather is cool, wet, and cloudy, since, under such conditions, it is frequently impossible for the grower to keep his plants dry.

Many of the varieties of the carnation grown commercially show marked susceptibility to the leaf spot, while certain varieties have been observed to be resistant. Both Red and White Matchless have been reported from various States as being highly susceptibile. In addition to these varieties, Gregory (5), in Indiana, has found the disease to be extremely severe on Betty Lou and Spectrum. Massey (6), after making observations during an epiphytotic on Long Island in 1928, listed Matchless, Eldora, Rosalind, Scepter, and Spectrum as susceptible and the Enchantress varieties as resistant. The writers during the last 3 years have found the variety Sophelia to be very susceptible, while Spectrum Supreme, Salmon Spectrum, Potentate, and Patrician were found to be moderately susceptible.

SYMPTOMS

Symptoms exhibited by the several carnation varieties susceptible to the bacterial leaf spot are fairly constant except for slight variations in color. The necrotic spots resulting from infection (Fig. 1 A and B) are sunken and of a more or less oval shape with the long axis of the leaf. The centers of the lesions in reflected light are pale brown with purplish concentric zonations, while the outer zone is water-soaked and yellow in transmitted light. Under conditions of high humidity the bacteria may ooze out on the surface of the lesion in a small drop. The spots average 8–12 mm. in length and may include the entire breadth of the leaf. With numerous centers of infection on a single leaf, the spots tend to coalesce.

Several spots ultimately cause the death of a leaf. This killing begins with a yellowing that occurs in advance of the water-soaked margin about the older lesions. The leaf gradually turns a straw yellow, withers, and dies, while the spots retain a rusty brown color.

The progress of the disease on a plant is from the lower leaves upwards. Symptoms always have been observed to appear first on the older leaves under both field and greenhouse conditions. While the bacterial leaf spot is, primarily, a foliage disease, it should be pointed out that in rare cases natural infections have been observed on the stems and flower buds, as well. No evidence has been obtained, however, that the disease is systemic. In cases of severe infection the plants may be killed. In those instances where the infection is less severe flower production may be arrested or curtailed for a considerable length of time.

PATHOGENICITY

Numerous inoculation experiments have been conducted with 3 different isolates of *Phytomonas woodsii* on several varieties of carnations, but, since

100 per cent infection occurred in all cases where conditions were favorable, the data are not presented. The first symptoms of the disease appeared approximately 3 to 4 days after inoculation. In no case, however, was infec-

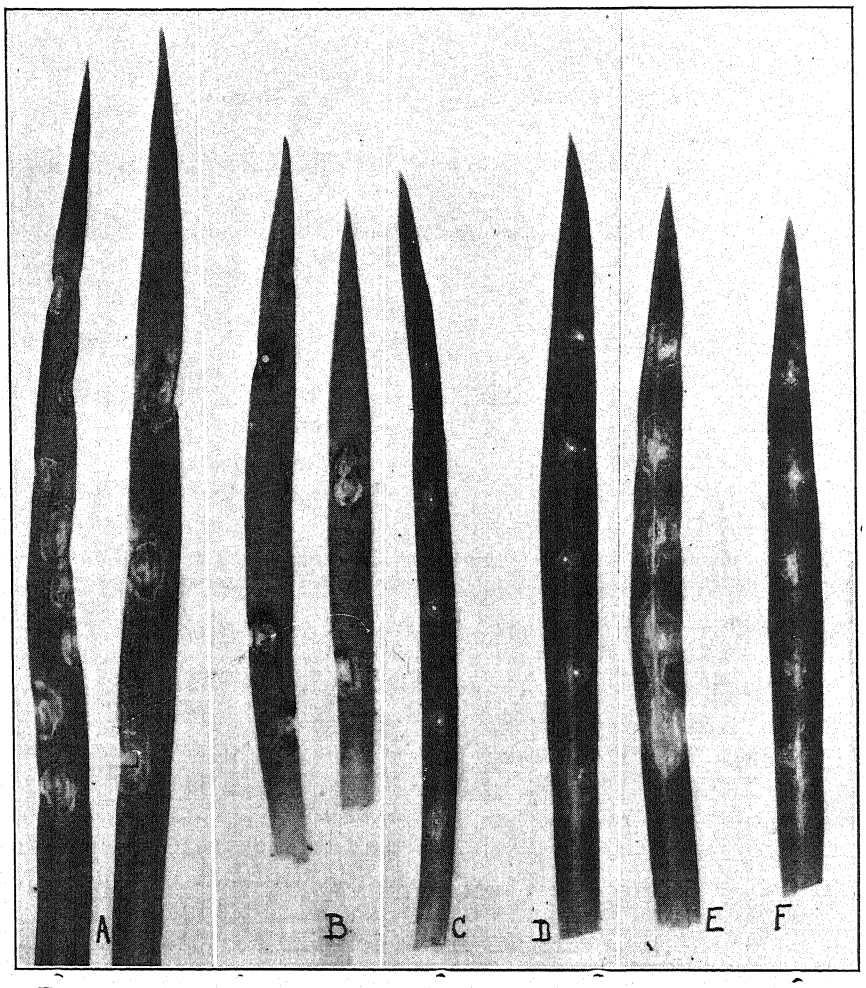


FIG. 1. A and B. Leaf spots resulting from natural infection with *Phyt. woodsii* on the varieties Spectrum (A) and Potentate (B). C to F. Effect of temperature on the development of bacterial leaf spot on leaves of the variety Sophelia. C, 60°; D, 65°; E, 75°, and F, 80° F.

tion obtained without wounds. This finding is at variance with the statements of Woods (10) and Smith (7) who claim that infection can be obtained in the absence of wounds, the bacteria presumably gaining entrance through the stomata. A strenuous, but unsuccessful, attempt has

been made to duplicate the results. Bacteria have been atomized and painted on unwounded leaves, and in some instances the bloom was rubbed free from the treated leaf. Both old and young leaves have been inoculated. Plants under test have been left in a moist chamber several days before and after inoculation. Variations in greenhouse temperature also have been utilized. In 4 experiments, drops of a bacterial suspension have been maintained for at least a week on the leaves of plants under bell

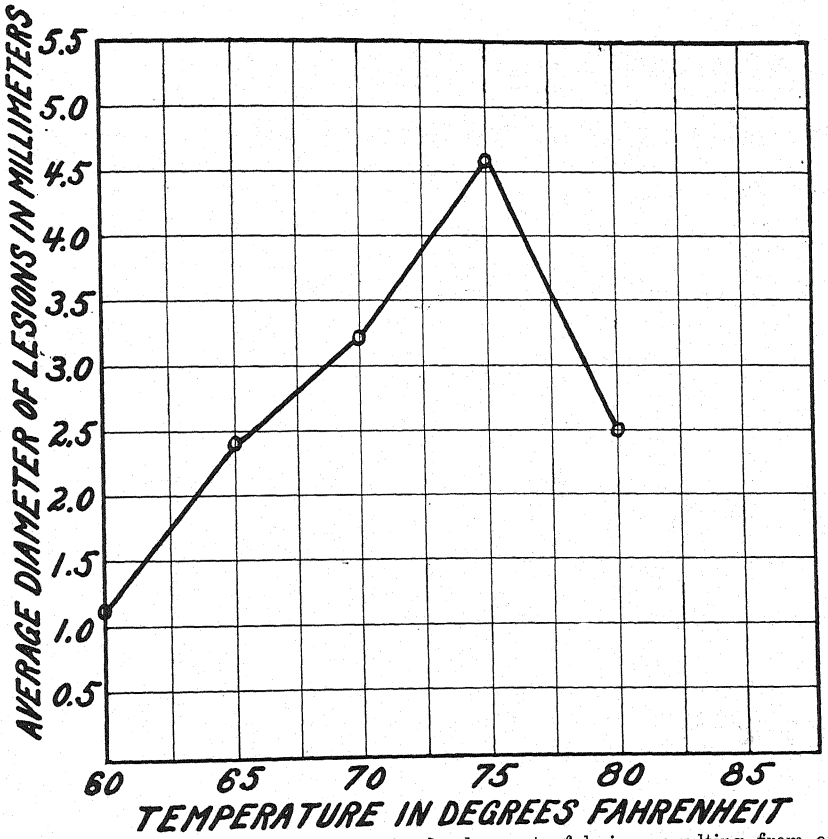


FIG. 2. Effect of temperature on the development of lesions resulting from artificial inoculation of the varieties Sophelia and Laddie.

glasses, but with negative results. In comparable series of experiments using plants whose leaves had been slightly wounded, infections resulted in all cases. It would appear, then, that under the conditions of these experiments infection with *Phytomonas woodsii* does not occur in the absence of wounds.

The feeding punctures and injuries of such common carnation pests as red spider mites, thrips, and aphids under natural conditions doubtless

offer a means of entrance for the bacteria. In 2 experiments conducted in the greenhouse, carnations of the variety White Matchless, free from the bacterial spot but infested with red spider mites, were grouped around 3 plants heavily infected with the disease. All of the plants then were subjected to frequent overhead watering, with the result that infection was soon evident on the spider-infested plants. In another experiment, however, bacteria in a water suspension were painted upon spider infested leaves of White Matchless with negative results. It seems reasonable to assume, nevertheless, until further evidence is obtained, that under natural conditions the bacteria gain entrance chiefly through wounds.

The effect of air temperature on the rate of development of the bacterial-spot lesions (Fig. 1, C to F) has been studied in a series of 3 experiments. Vigorously growing plants of the varieties Sophelia and Laddie were used. Five inoculations were made on each of 3 leaves of 4 plants, making a total of 60 inoculations. These were accomplished by puncturing the epidermis of the leaves with a sterilized needle and then painting the wounds with a water suspension of the bacteria. The diameter of the lesions was measured 15 days after inoculation and the data from the 3 experiments were summarized (Fig. 2). Development of the lesions was relatively slow at 60° F., with a rapid increase at the higher temperatures to the optimum at 75° F.

THE PATHOGEN

In describing the pathogen, 3 isolates were used. One was isolated in September, 1931, from carnation leaves of the variety White Matchless, collected at Skaneateles, New York. A second was a similar isolate, but reisolated in March, 1932; and the third was isolated in July, 1932, from specimens of the variety Sophelia, collected on Long Island. All 3 isolates behaved similarly.

Following is a description of the pathogen in culture:

Phytomonas woodsii is a small rod with rounded ends or at times pointed, occurring singly or less commonly in pairs. Spores are not formed, but frequently a central volutin granule is observed. A culture 48 hours old on beef extract agar (pH 6.85) grown at 27° C. showed the following cellular measurements: 1.56 μ (1.05–2.10 μ) by .67 μ (.47–1.05 μ). A Congo red negative stain was used in this case. A direct stain no doubt would give smaller measurements.

The organism is motile, having one or more polar flagella; is Gram-negative, and stains readily with carbol fuchsin. It is aerobic.

On beef extract agar (pH 7) at 27° C., growth is slow and the colonies are small, circular, and colorless. On slants, growth is scant, filiform, and somewhat creamy by transmitted light. Its consistency is butyrous.

Growth on potato-dextrose agar is more abundant and glistening. Brom-cresol-purple milk becomes alkaline (bluish), but no other change takes place. There is slight growth in Uschinsky's solution and in Clara's solution (4), but there is no green fluorescence. No liquefaction occurs in gelatin. Light hydrogen sulphide production is found in lead acetate agar. Ammonia is produced in beef-extract bouillon. Hansen's method of determination was followed. Nitrates are not reduced to nitrites. The method used was that of sulfanilic acid and a naphthalamine in acetic acid. The pathogen, when grown in tryptophane broth and determination made at the end of 1 and 4 days with the Ehrlich-Böhm test, showed no indication of indol production.

The ability of the pathogen to ferment carbohydrates was determined by methods employed and described in a previous publication by one of the writers (3). In the synthetic medium used, growth was slow, as is true on most media. Growth with a change in the hydrogen-ion concentration of the medium occurred with the following carbohydrates: dextrose, levulose, galactose, arabinose, xylose, rhamnose, lactose, glycerol, mannitol, acetic acid, citric acid, malic acid, and succinic acid. No growth occurred in sucrose, maltose, salicin, cellulose, lactic acid, and formic acid. Starch is not hydrolyzed.

CONTROL

Experiments on the control of the disease have not yet been conducted by the writers, but certain observations in ranges where carnations are being grown commercially have indicated promising leads. Many New York State florists have obtained benefit from the application of sulphur dust, while Gregory (5) in Indiana reports control of the bacterial leaf spot with 2 applications of a zinc sulphate spray. In several cases rapid spread of the disease has been stopped by fumigating rather than syringing for the red-spider control. General sanitary measures and maintenance of the greenhouse temperature and humidity as low as possible in the early fall also are advisable.

SUMMARY

The bacterial leaf spot of the carnation caused by *Phytomonas woodsii* is described and its economic importance discussed. Observations are made on the varietal susceptibility of the carnation to the pathogen. The pathogenicity of the causal organism, especially its relation to temperature is treated and illustrated. A description of *Phyt. woodsii* and its biochemical reactions are given.

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COLLETOTRICHUM TRUNCATUM (SCHW.), N. COMB., ON GARDEN AND LIMA BEANS

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(Accepted for publication July 17, 1934)

Specimens of Lima-bean stems and pods, bearing what appeared on casual examination to be a species of Vermicularia, have been collected by the writers from various sources in the Southeastern United States since 1931. In addition to severe russeting or scarification, the pods and stems bear numerous black pycnidia-like bodies that, on closer examination, prove to be sporodochia of a typical Colletotrichum, with curved spores produced among numerous dark brown setae. In January, 1934, specimens on Lima-bean pods, together with a pure culture of the fungus, were received from T. D. Persons, of the State Plant Board of Mississippi, through Miss A. E. Jenkins, of the Division of Mycology and Disease Survey. Since this material was accompanied by a request for identification, the writers have sought to trace the history and affinities of the organism and have made such emendations to earlier descriptions as seemed desirable.

The fungus has been found identical with one described by Schweinitz in 1832 as *Vermicularia truncata* Schw. on stems of *Phaseolus* sp. in Pennsylvania. Schweinitz's diagnosis was as follows:

"Peritheciis lineam vix excedentibus, nigris, (in leguminibus majoribus), adnatis, confertis, hemisphaericis, horizontaliter truncatis in majoribus; in minoribus irregulariter conico-truncatis, rugulosis. Setis filiformibus praesertim truncaturam occupantibus, divergentibus, longioribus, brevioribus immixtis. Saepe setae caducae sunt, ita ut perithecium orbatur.

Hab. in caulibus, sarmentis ac leguminibus Phaseolorum horti frequens, Bethl."

An examination of a portion of the original Schweinitz specimen in the Michener Collection, now in the Mycological Collections of the Bureau of Plant Industry, showed that the host probably was a Lima bean. No spores were found in several mounts made from the limited amount of material available, and Schweinitz gave no spore description. The general appearance and dimensions of the sclerotial bodies ("perithecia"), however, conform to the senile sporodochia on the Lima-bean pods from Mississippi; and also the abundance, pigmentation, and dimensions of setae are quite identical to those on the Lima-bean pods and likewise to those on bean stems artificially inoculated with the fungus sent by Persons.

The same fungus was described by Cooke in 1883 as *Vermicularia polytricha* Cke., based on a specimen collected in South Carolina (Ravenel's

F. Amer. Exs. 532). Cooke's diagnosis is as follows:

"Erumpens, cupulaeformis, strigosa, atra. Setis dense congestis, erectis, rigidis, subulatis, glabris, atrofuscis. Sporis lanceolatis, lunatis, nucleatis, hyalinus, (.03 × .004 mm.).

On Lima bean, (Rav., 2988). S. Carolina."

A comparison of the original specimens described by Schweinitz and by Cooke, together with a comparison of the author's descriptions, leaves little doubt that the two collections are nearly or quite identical, and are identical also with the recent collection from Mississippi.

The same fungus was again described by Heald and Wolf in 1911 as *Colletotrichum caulicolum*. Their description was based on material from living stems of the common garden bean, *Phaseolus vulgaris*, from Texas. Their diagnosis is as follows:

"Acervulis sparsis, nigris, lenticularibus, 150–250 μ. Setis copiosis, brunneis, septatis, utrimque rotundatis vel superne acutis, 60–120 × 3.5–4 μ; basidiis 30–60 μ, cylindraceis, hyalinis, plerumque 1–2 septatis; conidiis falcatis, hyalinis, granulosis, 18–30 × 3.5–4 μ."

In supplementary remarks the fungus is said to be the cause of a destructive disease of the Kentucky Wonder bean, and, according to their description, the symptoms correspond to those produced on bean plants inoculated with the Mississippi culture. Unfortunately, no specimen of the original collection by Heald and Wolf is available for direct comparison, but there appears to be little reason to doubt that the fungus is identical with that described by Schweinitz and by Cooke, as well as with the Mississippi specimen.

As stated previously, the fungus is undoubtedly a true Colletotrichum with spores formed externally among numerous setae. The numerous dark sclerotial bodies suggest a pycnidial fungus, when observed without proper magnification, but no true pycnidia have been observed in any of the specimens.

In view of the above facts a new combination appears to be warranted and the following is proposed, together with a modified and amplified version of the description by Schweinitz:

Colletotrichum truncatum (Schw.), n. comb.

Vermicularia truncata Schw. Syn. Amer. bor. No. 1865 (Published 1832).

Vermicularia polytricha Cke. Grevillea 12: 24. 1883–84.

Colletotrichum caulicolum Heald and Wolf. Mycologia 3: 10. 1911.

Acervulis lineam vix excedentibus, nigris, (in leguminibus majoribus), adnatis, confertis, hemisphaericis, horizontaliter truncatis in majoribus; in minoribus irregulariter conico-truncatis, rugulosis; setis copiosis, variabilibus, filiformibus praesertim truncaturam occupantibus, divergentibus,

longioribus, brevioribus immixtis, 60–300 × 3.5–8 μ; saepe setae caducae sunt, ita ut sclerotium orbatur; conidiis falcatis, lanceolatis, hyalinis, 18–30 × 3–4 μ.

On stems and pods of *Phaseolus lunatus* and on stems of *P. vulgaris* in Southern and Eastern United States.

In addition to the material sent from Mississippi by Mr. Persons, the Mycological Collections of the Bureau of Plant Industry now include por-

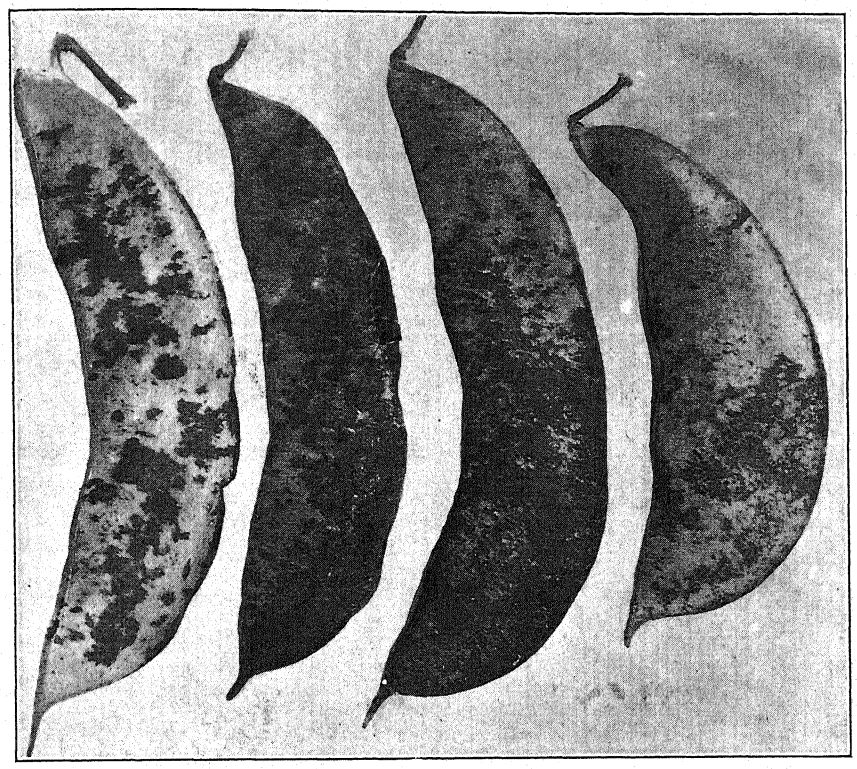


FIG. 1. Early stage of infection by *Colletotrichum truncatum* on Lima-bean pods.

tions of both the Schweinitz and Cooke specimens and also several collections that have been identified by various individuals as either *Vermicularia truncata* or *V. polytricha.*

The disease caused by this species of Colletotrichum, although not referred to in any known compendium of plant diseases, is by no means rare or local in distribution. Communications from pathologists in the Southern States indicate that it is not uncommon on Lima beans and is occasionally of some importance. In 1931 a field of 20 acres of Lima beans was found at Goldsboro, North Carolina, with about 5 per cent of the

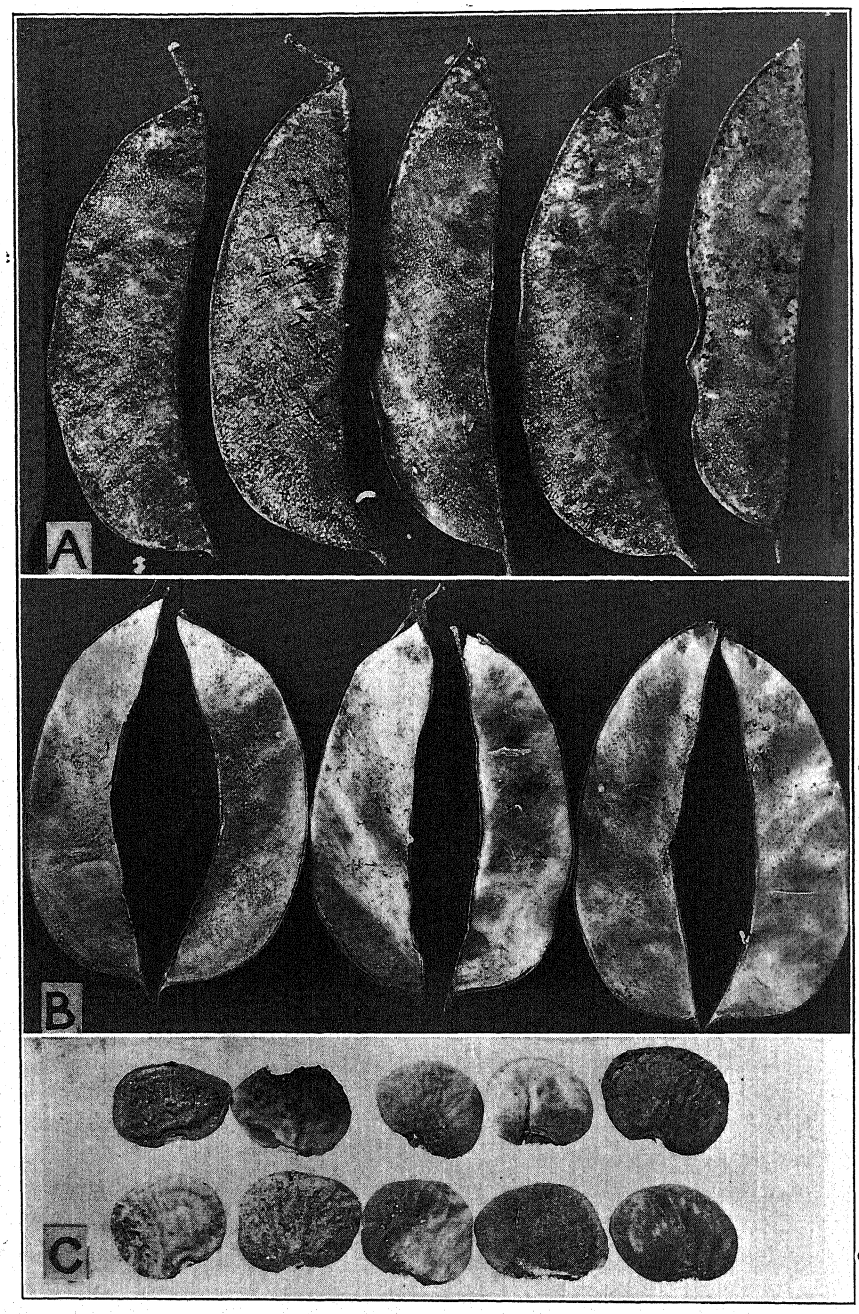


FIG. 2. A. Advanced stage of infection by *Colletotrichum truncatum* on Lima-bean pods, showing setose fruiting bodies superficially resembling pycnidia. B. Fruiting bodies on the inner side of pods. C. Type of injury on Lima-bean seed, with formation of sclerotial bodies on the surface.

plants heavily infected. During the same season 2 other infected fields were found at Norfolk, Virginia, and numerous specimens were collected in stores at Fort Gaines, Georgia. Collections from Pennsylvania, South Carolina, Georgia, Louisiana, Texas, and Mississippi attest to its fairly wide distribution in the Southern and Eastern United States.

The initial stages of infection are seen first on the pods as small reddish blotches (Fig. 1), which may spread over the entire surface. Definite lesions are not found. In later stages of development the infected areas become light brown or grayish, and numerous "perithecia" may be observed macroscopically (Fig. 2, A). The organism also infects the plant branches, girdling and killing them in much the same way as does pod spot (*Diaporthe phaseolorum*). In many cases the dry infected pods will show numerous "perithecia" on the interior of the shells (Fig. 2, B), and not infrequently the seed in such pods will likewise show these sclerotial bodies over the surface (Fig. 2, C). It appears, therefore, that the fungus may be spread through infected seed.

Natural occurrence of the fungus on garden beans is known only from the description by Heald and Wolf. Artificial infections, however, have been produced on 18 varieties of *Phaseolus vulgaris*,—the symptoms ranging from slight scarification of stems and leaves to very severe stem injury accompanied by defoliation. Artificial infections on varieties of *P. lunatus* indicate that the latter species is more completely susceptible than *P. vulgaris*.

Colletotrichum truncatum is entirely distinct from the common bean anthracnose organism, *C. lindemuthianum*. The characters in which it differs most conspicuously from *C. lindemuthianum* are the curved, lanceolate spores and habit of growth in culture. The types of injury produced by the 2 species on beans also differ conspicuously.

The authors wish to acknowledge the assistance given by Dr. W. W. Diehl, of the U. S. Department of Agriculture, in the examination of herbarium specimens and in the preparation of the manuscript.

BUREAU OF PLANT INDUSTRY,
U. S. DEPARTMENT OF AGRICULTURE.

SOME EFFECTS OF PHYMATOTRICHUM ROOT ROT ON THE MICROSCOPIC CHARACTERS OF COTTON FIBERS[1]

G. N. Stroman, J. J. Taubenhaus, and Walter N. Ezekiel

(Accepted for publication April 26, 1934)

The cotton root rot, caused by the fungus *Phymatotrichum omnivorum* (Shear) Duggar, kills cotton plants at various times during the growing season. Plants attacked early in the season produce only occasional immature bolls, while those that succumb later may already have matured a large part of their crop. In separate papers, some of the writers have discussed the relative yields obtained from plants that succumbed to root rot at different times during the season,[2] and also the effect of the death of plants from attack by root rot at the various times on the quality of lint and seed produced.[3] The present paper reports the results of microscopic examination of fibers from these same cotton plants.

MATERIAL AND METHODS

The material studied consisted of thirteen lots of cotton lint from a single field of Startex cotton grown under irrigation at College Station, Texas, in 1931. Of these lots, 11 were from plants that had died or had shown advanced symptoms of Phymatotrichum root rot at various intervals during the season of 1931; while 2 lots were from plants that were still apparently normal on September 10, when the first picking was made. A random sample was taken from each lot of cotton for microscopic study of the fibers. From the sample a tuft of fibers was mounted in glycerine in parallel fashion on a microscope slide and a cover glass placed on top. Thirty individual fibers from each lot of cotton were measured as to ribbon width, thickness, and number of convolutions or natural twist. According to Pope,[4] more than 100 fibers should have been measured. This was not possible in the present work, but the results given below are treated so as to show their statistical significance.

The ribbon width is the measurement taken across the flat surface of the fiber. It was taken at the widest point or points of the fiber. The thick-

[1] Approved for publication by the Director of the New Mexico Agricultural Experiment Station and by the Director of the Texas Agricultural Experiment Station as Technical Contribution No. 296.

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TABLE 1.—*Means, standard deviations, and ranges in measurements of microscopic characters of cotton fibers, from plants that succumbed to root rot at various periods during the growing season. Averaged from measurements of 30 individual fibers, in each lot*

Lot No.	Interval during which plants succumbed to root rot	Width of fibers, in microns			Thickness of fibers, in microns			Twist, convolutions per 1.6 mm. of fibers		
		Mean	Standard deviation	Range	Mean	Standard deviation	Range	Mean	Standard deviation	Range
1	to June 30[a]	21.41	4.91	8.7–29.0	11.24	3.47	4.2–18.8	11.73	2.21	8–16
2	July 1 to July 7[b]	21.90	4.23	14.4–32.0	11.55	2.07	8.7–17.4	11.53	3.56	6–20
3	" 8 to " 15	26.74	4.34	20.3–36.4	14.87	3.62	8.7–21.8	8.93	2.91	5–17
4	" 16 to " 22	26.77	5.01	15.9–37.7	15.45	3.39	10.2–24.5	9.37	1.91	7–14
5	" 23 to " 30	24.54	4.59	15.9–33.4	13.48	3.66	7.2–21.8	10.50	3.42	7–16
6	" 31 to Aug. 4	23.38	3.34	17.4–29.0	13.14	2.62	8.7–18.8	10.43	2.51	6–16
7	Aug. 5 to " 11	22.03	3.60	14.4–29.0	12.34	2.50	8.7–17.4	14.60	2.94	10–20
8	" 12 to " 18	21.65	3.05	14.4–27.5	12.04	2.29	8.7–17.4	14.80	2.75	10–20
9	" 19 to " 25	20.82	3.55	11.4–29.0	11.85	2.35	8.7–20.3	14.03	3.41	10–22
10	" 26 to Sept. 3	20.84	3.00	14.4–29.0	11.57	2.35	7.2–15.9	13.03	4.14	5–20
11	Sept. 4 to " 10	21.41	3.66	14.4–30.5	12.02	3.22	7.2–20.3	15.20	4.16	7–25
12	Normal plants	21.16	3.55	12.9–29.0	11.51	2.35	5.7–15.9	13.27	2.63	9–22
13	Normal plants	23.02	3.13	18.8–29.0	11.95	2.26	8.7–17.4	14.13	2.78	7–18
	Average of normal plants	22.09			11.73			13.70		

[a] Only 8 bolls were borne by the 77 plants of this group; 2 of these bolls, yielding 1.9 gm. of the 6.6 gm. of lint cotton, were borne on plants that recovered and were still green on September 10.

[b] The single boll harvested from the 49 plants in this group was borne by a plant that recovered and was green on September 10.

ness was measured at small places on the fiber between two of the convolutions. However, due to the twisting of the fibers, this measurement probably was greater than the actual thickness of the fibers. The thickness and width of fibers were measured with a micrometer eye piece. The width and thickness were taken at 5 different places on each fiber and averaged. The convolutions or twists of the fiber were obtained by counting the number of twists along about 1.6 mm. of the cotton fiber, that is, across the low-power field of vision.

RESULTS

The mean, standard deviation, and range of the measurements of the fibers are summarized in table 1. In general, the fibers from plants that succumbed to root rot between July 8 and August 4 showed distinct differences in microscopic characters of fibers from plants that succumbed later in the season or from the normal plants. With lot 1, part of the crop, and with lot 2, all of the crop were from plants that had recovered from the disease, and the characters of the lint from these lots were, in general, similar to those of the normal plants.

Ribbon Width of Fiber

The mean width of fibers in lot 3 was $26.74 \pm .53\mu$, and of fibers from the normal plants of lot 12 was $21.16 \pm .44\mu$. The difference between these two lots was $5.58 \pm .69\mu$, a significant difference. The values for lot 4 were practically the same as for lot 3. The difference between the mean widths for lots 5 and 12 was $3.38 \pm .71\mu$. Similarly, lot 6 differed from lot 12 by a fairly significant figure of $2.22 \pm .60\mu$. The measurements for the two lots of normal plants, while not identical, were not greatly different; the difference amounting to $1.86 \pm .58\mu$, barely 3 times its probable error.

Thickness of Fiber

The fibers of lots 3, 4, and 5 were significantly thicker than those from the normal plants. Lots 3 and 13 differed by $2.92 \pm .53\mu$. As noted above, the thickness as measured was perhaps not precisely indicative of the actual thickness of fibers. The recorded thicknesses for lots 3, 4, and 5 were, therefore, possibly biased by the smaller number of twists in fibers of these lots.

Convolutions or Natural Twist of Fiber

Lots 3, 4, 5, and 6 were again distinct from the other lots in the frequency of twisting. They averaged fewer twists per unit length. Of these 4 lots, the one with the larger number of twists was lot 6, with $10.43 \pm .31$ twists per 1.6 mm. of fiber. Comparing this with lot 12, which

had fewer twists of the 2 lots of normal plants, 13.27 ± .32 twists, there is a difference of 2.84 ± .45 twists, over 6 times its probable error. In this character, lots 1 and 2 were intermediate between these lots mentioned (3, 4, 5, and 6) and the normal plants. Lot 2, with 11.73 ± .27 twists, differed from lot 12 by 1.54 ± .42 twists, a fairly significant difference, since it is 3.7 times its probable error.

DISCUSSION

In general, the cotton fibers from the lots of cotton that died early in the season (excluding lots 1 and 2) had microscopic characters significantly different from those of the normal cotton fibers. It may be deduced that these less twisted, coarser fibers from plants killed early in the season by root rot probably would have a lower spinning value than normally developed fibers and would be more likely to be discarded as waste in the spinning. These results explain the inferior quality and strength of yarns spun in some earlier work,[5] from lint of cotton plants killed early in the season by root rot, as compared to yarns from cotton from normal plants or from those attacked later in the season.

In the absence of some experimental evidence bearing on these relations, any attempt to explain the excess width and thickness of fibers from plants that succumb to root rot early in the summer must remain completely hypothetical. The abnormal characters of the fibers may result from the effects of translocation of soluble materials of some sort upward from the areas invaded by the fungus, prior to the final death of the plant; or of materials from the stem, which may remain green after the leaves are shed; or from environmental influences on the fibers in the bolls during the month intervening between death of the plants and picking.

It is of considerable importance that the apparently undesirable type of fiber did not constitute a very large proportion even of the material harvested from plants with root rot in the experimental work under consideration. As shown in detail in another paper of this series,[5] the greater losses in yield occurred with plants succumbing to root rot early in the season. Of the cotton picked from plants that had succumbed to root rot, slightly more than 90 per cent came from plants that had died in the 5 weeks immediately preceding harvest; that is, during the period in which the microscopic characters of the fibers were apparently not significantly affected by death of the plants from root rot. Less than 10 per cent of the yield from these root-rot plants in the College Station experiment was obtained from the plants that succumbed to the disease during the preceding 4 weeks, during which evident changes of the fibers apparently were associated with root-rot injury.

[5] Campbell, M. E., and H. H. Willis. Spinning test of root rot cotton (Texas—crop of 1927). U. S. Dept. Agr. Bur. Agr. Econ. 1928. (Mimeographed).

However, the continued irrigation in the experimental field encouraged continued spread of root rot during the season. Under dry-farming conditions, during many seasons,[5] the spread is, instead, lessened with the advent of dry summer weather. Therefore, of the plants with root rot, a higher proportion succumb earlier in the season, during the period when perceptible injury to the quality occurs. It follows that under dry-farming conditions more than 10 per cent, although probably less than 20 per cent, of inferior fibers might be expected in the cotton picked from plants that succumb to root rot. Thus, in a field in which as much as half of the crop came from plants that had succumbed to root rot, 5 to 10 per cent of the crop might consist of such inferior fibers; while if only 10 per cent of the crop had been harvested from plants with root rot, no more than 1 or 2 per cent of inferior fibers would be expected. In the exceptional cases in which the majority of cotton plants in a field have succumbed to root rot early in the season, it is obvious that the small crop that could be harvested would consist largely of the inferior fibers.

SUMMARY

Microscopic studies have been made of cotton fibers from plants killed by root rot at different periods between June 30 and September 10, 1931. In each sample, 30 fibers were measured as to ribbon width, thickness, and number of convolutions per unit of length. The mean, standard deviation, and range of individual fibers in each lot are tabulated. Fibers from plants killed early in the season were wider and thicker and had a smaller number of convolutions per unit length than fibers from normal plants. Fibers from plants killed later in the season were not significantly different from those from the normal plants.

It is pointed out that the coarser, less twisted fibers from plants succumbing early in the season constituted less than 10 per cent of the cotton harvested from the root-rot plants grown under irrigation in this experiment, and would probably average not more than 20 per cent of the crop from plants that succumb to root rot under dry-farming conditions. (New Mexico and Texas Agricultural Experiment Stations, cooperating. Agricultural Experiment Station, Las Cruces, N. Mex., and College Station, Texas).

[6] *Loc. cit.*

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VARIABILITY OF MONOSPORIC CULTURES OF COCCOMYCES HIEMALIS[1]

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INTRODUCTION

The literature dealing with variation in fungi is rather extensive. Nearly all of the investigations in this field have been reported within the past 40 years. The conception of the fixity of a species, dominant until the latter part of the last century, probably accounts in large part for the fact that fungus variability was not studied earlier.

Schroeter (38) in 1879, working with certain rusts on Carex, was one of the first to observe that there were physiological forms or, as he termed them, "sister species." The first important demonstration of this phenomenon was made in 1894 by Eriksson (14) in his cross-inoculation experiments with *Puccinia graminis* Pers. on several species of grain. Ward (47) in 1902 found this pathogenic variation in *Puccinia dispersa* (Eriks.) Syd. on bromes. Between 1902 and 1903, Neger (32) in Germany, Marchal (28) in France, and Salmon (37) in England, reported pathogenically distinct forms for some of the powdery mildews on grasses and grains. The phenomenon of heterothallism in the mucors was demonstrated in 1904 by Blakeslee (2).

The work of these investigators opened up the importance and possibilities of the study of fungus variability and ushered in a series of investigations that have firmly established the fact of variation within a fungus species for fungi representing several families in both the ascomycetes and basidiomycetes. It is difficult to name a parasitic fungus in which variability has been sought but not found. It is now coming to be the common expectation to find much variation between different isolations of any fungus species.

The work on fungus variation has been directed mainly toward patho-

[1] Approved for publication by the Director of the Wisconsin Agricultural Experiment Station.

[2] The writer is particularly grateful to Dr. G. W. Keitt and to Dr. B. M. Duggar for their helpful advice and criticisms during the course of the investigation and the preparation of the manuscript.

genic fungi showing physiological specialization on their host plants. The knowledge gained by the study of physiological specialization has proved very important from several standpoints. As pointed out by Stakman (41), this knowledge is essential to the development of varieties of plants resistant to pathogenic fungi; to the understanding of the epidemiology of a disease; and to the establishment of judicious and adequate plant quarantines.

Work on fungal variation has also been done with saprophytic fungi and certain pathogenic ascomycetes and imperfects that exhibit their variability only in culture. The study of fungal variation in culture has proved useful in stimulating interest and study in the genetics of fungi; in providing a means for the study of sex in fungi; in mycological taxonomy; and sometimes in giving the first clue to possible host specialization.

There is some disagreement as to the mechanism by which heritable variations arise. Various workers have attributed this phenomenon to 1. Mutation, Stakman and co-workers (42), Brown (4), and Stevens (44); 2. Hybridization, Stakman and co-workers (42); 3. Segregation from a heterogeneous parent or heterokaryosis, Hansen and Smith (17) and Brierley (3); 4. "Dauermodification," Jollos (22) and Caldis and Coons (6); and 5. Dissociation from cyclic phases, Leonian (27). The difference in the theories as to the nature and cause of variability may be partly due to the fact that their sponsors have worked with different types of organisms and have used different procedures in studying their variation.

The purpose of this investigation was the study of variations exhibited by monosporic cultures of *Coccomyces hiemalis* Higgins, the fungus causing the leaf spot disease of sweet and sour cherries. An investigation of the epidemiology and control of this disease has been in progress for several years at Wisconsin. A study of the variability of this fungus seemed desirable to further the knowledge of the epidemiology and control of the disease, to initiate a study of the physiology of the pathogen, and to contribute to the foundation for further inquiries into the physiology of its parasitism.

Variability in the monosporic cultures was sought in their morphology, physiological reactions, and pathogenicity on several species of Prunus.

PHYSIOLOGICAL STUDIES

Methods and Materials

Monosporic cultures of *Coccomyces hiemalis* were isolated by the method described by Keitt (23) from sour cherry material obtained between 1930 and 1932 from Oregon, New York, New Jersey, Pennsylvania, Michigan, and several localities in Wisconsin. The cultures were carried on a medium

consisting of a water extract of 100 grams of cracked Lima beans, 10 grams of agar, and one liter of distilled water.

The cultures were numbered in the order in which they were isolated: Isolate Nos. 1–66, 86–90, and 103–113 from Wisconsin; 67–82 and 97–101 New York; 83–85 Michigan; X1–X59 Oregon; 91 Pennsylvania; and 92 New Jersey.[3] All isolates are from single ascospores, except 1 and 36–66, which are from single condia. No difference was observed between monoconidial isolates as a class and monoascosporic isolates.

Stock cultures on Lima-bean agar were stored at 9° C.[4] Transfers were made every 2–6 weeks. When carried on Lima-bean agar with sufficient transferring, most of the isolates slowly lost their vigor. This lack of vigor was manifested in a slower growth rate, fewer spores produced, and perhaps, finally the loss of sporulation. To obviate this difficulty those cultures used more intensively in these studies were passed through the host at least twice during the course of the investigation. These monosporic reisolations differed from the cultures used for the inoculations only in an increased rate of growth, increased sporulation, and sometimes a darker shade of color.

In most of the studies on physiology a standard medium modified from Leonian (26) was used both as a liquid and a solid substrate. The medium contained:

KH_2PO_4	0.4 per cent	Dextrose	2 per cent
Peptone	0.4 per cent	Distilled water	
$MgSO_4$	0.05 per cent	pH 5.5	

In order to obtain good growth, 1 per cent of liquid malt extract was added to this in most of the work. 1.7 per cent agar was added to make the solid substrate. Cultures in the liquid medium were grown in 250 cc. Erlenmeyer flasks containing 50 cc. of the medium. Each flask was seeded by adding 2 drops of a heavy spore suspension obtained by scraping off the spores of 2-week-old Lima-bean-agar slant cultures into 5 cc. of sterile distilled water. Dry weights of the fungal growths were obtained, with a variation of 1.5 mg., by filtering the liquid culture through 7 cm. Schleicher and Schuell filter papers, No. 589, washing with water, and drying over night at 95°.

Petri dishes containing 25 cc. of agar were seeded by transferring small masses of spores from Lima-bean-agar cultures by means of a needle, giving a seeded area of 1–2 mm. in diameter. All cultures were incubated at 20° unless otherwise stated. All cultural comparisons were made of material incubated in dark control chambers.

[3] The writer wishes to thank Dr. M. P. Backus for several of these cultures.

[4] All temperatures noted in this paper are in degrees Centigrade.

Because of the very slow growth of the fungus, both liquid and solid media cultures were usually incubated for 6 weeks before final data were taken.

Colony Variations

Each isolate differed from other isolates in certain characteristics that varied greatly with the medium used, the temperature, and the age of the colony. When grown on the standard agar with malt extract for 4 weeks at 20°, the isolates could be classed according to their colony color as follows: 1, 11, 47, 52, 87, 91, and X25 as flesh pink; X2, X12, and 21 as reddish pink; 20 and 58 as buff; 5, 6, 17, 24, 101, X14, and X27 as light brown; 27, 29, 43, 45, 49, 66, 69, 76, 77, 83, 92, X53 as dark brown; 8, 30, and 90 as orange; 3 as black; and 7, 13, 105, 110, and X46 as white. These latter were white from the time of isolation from single ascospores. Some of the other isolates produced white sectors soon after being isolated, or when they were allowed to become stale in culture from lack of sufficient transferring. White sectors and white isolates produced no spores and never reverted to a sporulating or colored condition. Temporarily white colonies were produced by some of the isolates when grown on potato-dextrose agar.

When cultures, grown for 2 weeks at 20° on Lima-bean agar or on the standard agar, with or without malt extract, were placed at 4°–8° for 3 weeks, the following isolates produced a dark-colored or black colony and blackened the medium: 6, 17, 24, X14, 27, 29, 43, 45, 49, 66, 69, 76, 77, 83, 92, 101, X27, and X53. These are all classed as light or dark brown in color. The other isolates, excepting No. 3, were never observed to produce a dark-colored pigment.

Isolate 3 differed markedly from all other isolates in producing a black mycelium and darkening the medium during the first few days of growth at 20° C. in the standard liquid and solid media and on Lima-bean agar. The colonies of some of the isolates, listed as light brown and dark brown, developed black centers after 4–6 weeks when grown on the standard and Lima-bean agars at 20°. This blackening of the centers of the colonies was hastened at lower temperatures.

The isolates were observed to differ also in regard to the contour of the colony and the growth rate. Rate of growth is considered under a separate heading. Isolate 3 produced on agar a colony margin different from that of any other isolate in that the margin was composed of radiating hyphae. In all other isolates the colony margin was opaque due to the fact that the margin was abrupt and piled above the agar surface.

It was observed that the isolates were most distinctive in their colony characteristics at a temperature of 20° or 24°.

In the standard liquid medium with 1 per cent malt extract isolates 3, 43, 69, 92, and 101 tended to produce a mat-like growth, while 24, 27, 29, 30,

77, 83, 91, and X14 produced many small distinct colonies. This difference in growth might be explained by the fact that in every case the former isolates had a higher growth rate than those giving distinct colonies.

In this same medium after 4–8 weeks, it was observed that isolates 69, 91, and 92 gave a culture which, with the application of good suction, filtered very slowly through Schleicher and Schuell filter papers No. 589. With isolates 3, 6, 27, 30, 43, and X14 this difficulty was not encountered.

It was thought that the cause of the slow filtering might be the presence of a gum-like substance produced in the culture. Attempts to precipitate in alcohol such a substance from the filtrates of the slow-filtering cultures were unsuccessful. These filtrates had exactly the same viscosity as those that filtered easily, when tested with a pipette delivering 10 cc. of water in 1½ minutes.

Spore Production

Production of Conidia in Relation to Age of Culture. Lima-bean agar in uniform tubes and with a uniform slant was thoroughly seeded by pouring a concentrated spore suspension of each isolate used from tube to tube. The cultures were placed at 20°. At intervals graduated from 2 to 10 days, 2 tubes of each isolate were examined for spore production. This was done by introducing 3 cc. of water into each tube and thoroughly scraping the spores into suspension. After suitable dilution of the suspension, the number of spores per low power field of the microscope was counted in drops of the suspension on a slide. The drops were transferred with a wire loop 4 mm. in diameter. The average of 10 counts made near the center of each of 10 drops from the duplicate tubes was used for each determination.

Considerable variation was found between the isolates in the number of spores produced in the 2 series run. Six of the 9 isolates used in one series are represented in figure 1. Germination counts were made of the spores on 1.7 per cent agar plates after 48 hours at 20°. Spores of the various isolates gave from 92–98 per cent germination on the 8th day after seeding, from 69–98 per cent on the 32nd day, and from 40–98 per cent on the 42nd day. Isolates 30 and X14 gave the highest germination and isolates 83 and 27 the lowest.

Production of Conidia in Relation to Temperature. Lima-bean-agar slant cultures were prepared as described for the study of conidial production in relation to age of culture. The cultures were incubated at 20° for 6 days and placed in duplicate at the following temperatures: 4, 8, 12, 16, 20, 24, 28, and 30°. Three weeks from the time of seeding the cultures were examined, as described previously, for the number of spores produced. The 18 isolates tested in 3 series varied considerably in number of spores produced and in temperature relations for spore production. Six of the isolates varying most are represented in figure 2.

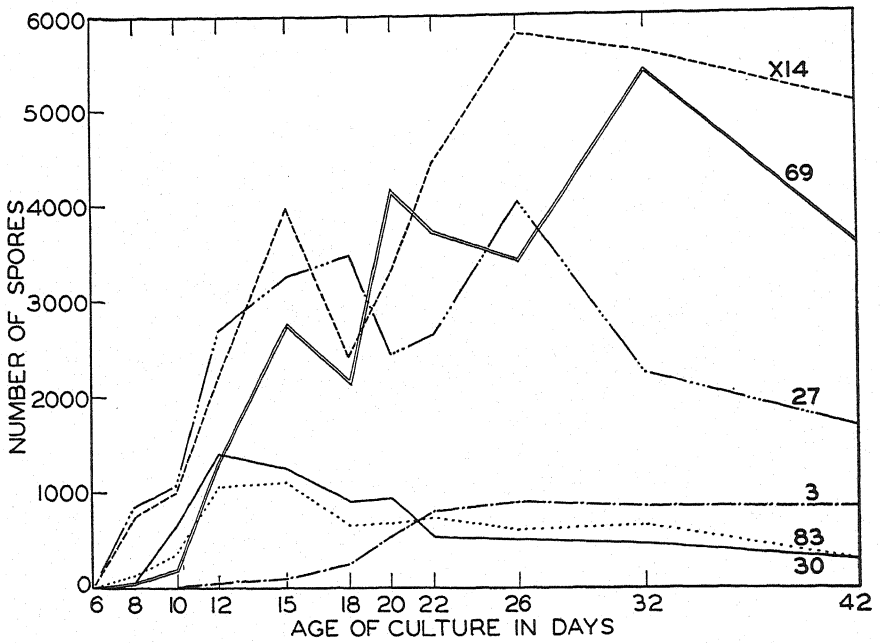


FIG. 1. The age of Lima-bean-agar cultures in relation to the number of conidia produced at 20° by 6 isolates of *C. hiemalis*.

Several isolates, including 1, 52, and X25, sporulated much more sparingly in culture than isolate 3 (Fig. 2).

Production of Microconidia. Since Craigie (7) demonstrated the functioning of the spermatia in certain rusts, the attention of mycologists has been drawn to similar bodies, microconidia, which are produced by so many of the ascomycetes. Tulasne, the first to describe these bodies, believed that sometime they would be demonstrated to be pollen-like in function. In a few instances microconidia have been shown to have a sexual function. Drayton (11) found two self-sterile races of *Sclerotinia gladioli* (Massey) Drayton, either of which, when "spermatized" by the microconidia of the other race, were interfertile and produced apothecia. Dowding (10) demonstrated the functioning of "oidia" in *Ascobolus stercorarius* (Bull.) Schroet.

Higgins (18) noticed the occurrence of microconidia on leaves parasitized by *Coccomyces hiemalis*. Keitt, while working with cultures of the fungus, commonly found microconidia produced in old Lima-bean-agar cultures. These bodies were not so commonly found by the writer, even when older Lima-bean-agar cultures developed at 20° were kept at 8–12° for several months.

In connection with studies on the relation of temperature to the growth

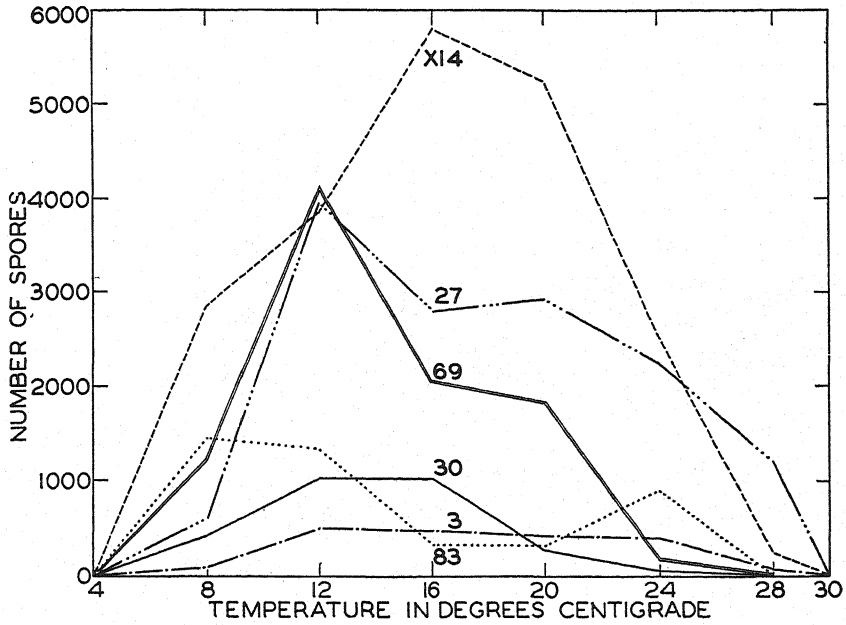


FIG. 2. Temperature in relation to spore production of 6 isolates of *C. hiemalis* grown on Lima-bean agar for 21 days. Cultures were incubated at 20° for the first 6 days.

of colonies on the standard agar plus 1 per cent malt extract, examinations were made at different periods for the occurrence of microconidia on colonies of the various isolates at the different temperatures. It was found that 3- to 5-week-old colonies of isolates 3, 30, 77, and X14 produced microconidia abundantly. Occasionally, a few microconidia were observed from isolates 83 and 91. More than 16 other isolates were never found to produce them under the stated conditions. These bodies were produced at temperatures from 8°–24°, 4° being too low and 28° too high for their production under the conditions of the experiment. Production of microconidia could not be correlated with any other characteristics of the isolates.

An attempt was then made to find microconidia on leaves parasitized by several isolates, including those producing these bodies in culture. Leaves bearing one- or two-month-old lesions were picked, washed, and put in Petri-dish moist chambers. Three days later they were again washed to remove the conidial horns and then replaced in the Petri dishes. They were kept at 12° for 3 days and then at 8° for 8 days, and examined for the production of microconidia. Lesions of all 12 isolates tested were found to contain few to many microconidia. Conidia were usually found in abundance also.

The microconidia of the various isolates varied little in size. Their

lengths ranged from 3.6 to 6.4 microns with an average of 4.9 and the width varied from 1.8 to 2.2 microns.

Germination of Conidia

Spores from 2- to 3-week-old Lima-bean-agar slant cultures were germinated at 20° for 48 hours. Ordinarily, spores germinate 100 per cent on 1.7 per cent agar plates with, or without, nutrient salts. Germination in drops of pure water on slides or in Van Tieghem cells is poor and erratic.

Lack of oxygen completely inhibits germination. Carbon dioxide given off by bits of crushed cherry leaves in Van Tieghem cells or Petri-dish moist chambers is toxic to the spores, but, if this gas is absorbed by the introduction of vessels containing barium hydroxide solution, normal germination results.

In the studies on the source of carbon it was desired to add the sugars after the basic liquid medium had been seeded and initial growth had taken place. Therefore the basic liquid medium in 250 cc. flasks was autoclaved separately in 2 series and seeded with conidia before the sources of carbon were added. It was found that little or no germination took place in the absence of the carbon source, whereas, in the full nutrient solution, germination proceeded normally.

The effect of sugars and some other compounds on germination was then studied in Van Tieghem cells, using conidia of isolates 69 and X14. The results of two series are presented in table 1. The drop of liquid placed in the bottom of each cell corresponded to the liquid in which the spores were suspended. About 100 spores per drop was found suitable. Since the two isolates gave similar results, the average of their germination counts is given.

The lack of germination in the absence of sugars, which was observed in 50 cc. of medium in a flask, could not be duplicated in Van Tieghem cells. Since the spores in the Erlenmeyer flask were at the bottom of the liquid, and in the cell were in contact with the air at the bottom of a drop, the discrepancy in results is probably due to the difference in the availability of oxygen.

The results of the germination tests shown in table 1 indicate that spores germinate better in an osmotic concentration as found in a balanced nutrient medium, that peptone and malt extract promote growth, that NaCl and aspartic acid are somewhat toxic, and that glycerine supports less germination than sucrose, under the condition of the experiments.

In the studies on the relation of hydrogen-ion concentration to germination, the medium used consisted of 1.7 per cent agar, 0.4 per cent KH_2PO_4, and 0.05 per cent $MgSO_4$ in distilled water. The hydrogen-ion concentration was adjusted by the addition of 1/5 normal HCl or 1/5 normal NaOH

TABLE 1.—*The effect of various organic and inorganic materials on germination of conidia of Coccomyces hiemalis in Van Tieghem cells*

Germination medium[a]	Germination after 48 hours	
	Average	Average length of germ tubes
	Per cent	*Microns*
Distilled water	23	19
City water	42	34
Solution of NaCl, 0.85 per cent[c]	28	15
NaCl, 0.85 per cent + sucrose, 2 per cent[c]	24	15
Pure sucrose, 2 per cent	77	43
Pure glycerine, 2 per cent[d]	28	20
Salt solution[b]	86	57
2 per cent sucrose + salt solution	92	61
2 per cent glycerine + salt solution	87	68
Peptone, 0.5 per cent	100	120
Glutamic acid, 0.5 per cent[c]	75	38
Glycine, 0.5 per cent[c]	42	26
Aspartic acid, 0.5 per cent[d]	0	0
Liquid malt extract, 2 per cent[c]	100	136

[a] Adjusted to pH 5.5 with $\frac{N}{5}$ NaOH and autoclaved 15 minutes.

[b] The salt solution containing 0.4 per cent KH_2PO_4 and 0.05 per cent $MgSO_4$.

[c] Used in one series only.

[d] Spores in these media appeared vacuolate.

and determined both before and at the end of the 48-hour period with the quinhydrone electrode. Determinations of pH were made on the cold agar. A drop of the spore suspension containing about 200 spores was placed on the agar surface. Agar plates were in duplicate, each plate being used for spore suspensions of 4–6 isolates.

At the end of the 48-hour period of germination, 4–6 drops of concentrated commercial (40 per cent) Formalin was added to each plate. By fixing the germinating spores in this manner, a large series could be run, and more accurate results obtained. To get the percentage germination at least 50 spores were counted in each drop and for the length of germ tubes at least 25 spores, giving at least 100 and 50 spores, respectively, for any one reading. The method of taking the readings follows: in each low-power field of the microscope the number of germinated spores and of ungerminated spores was recorded and the length of the germ tubes of each spore in the field was taken by means of a calibrated ocular micrometer. The per cent of germinated spores was calculated on the basis of the total number of spores observed and the length of germ tubes was based on the number of germinated spores used for the measurements.

The results of 3 series showed that the 6 isolates used had the same range of pH for spore germination, from pH 2.9 to 8.5. Spores germinated 100 per cent from about pH 4 to about pH 7. The curves for the average length of germ tube, however, had a distinct maximum at pH 5.3 to 5.6. The isolates showed some variability in the average length of germ tubes. The curves for the average length of germ tubes of 5 isolates of one series are plotted in figure 3.

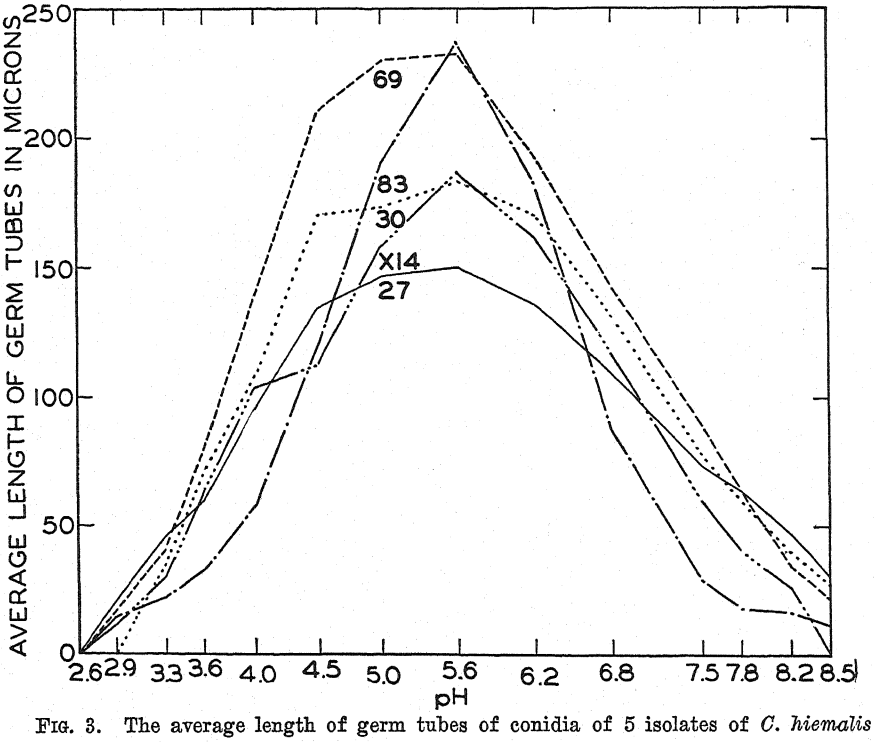


FIG. 3. The average length of germ tubes of conidia of 5 isolates of *C. hiemalis* germinated at 20° C. on agar buffered at various hydrogen-ion concentrations.

Growth Rate

Six isolates were grown in two series in a liquid medium for 12 weeks. The dry weight of the mycelium, total acidity, and pH were taken of duplicate flasks of each isolate every week. The medium contained:

KH_2PO_4	0.2 per cent	Trommer's malt extract	1 per cent
Peptone	0.1 per cent	Dextrose	2 per cent
$MGSO_4$	0.05 per cent	Initial pH about 6	

The hydrogen-ion concentration was obtained by the use of the quinhydrone electrode. The amount of growth differed between some of the isolates, but the type of curve was essentially the same for all (Fig. 4).

The change in hydrogen-ion concentration produced by the several isolates was not proportional to the corresponding amount of growth.

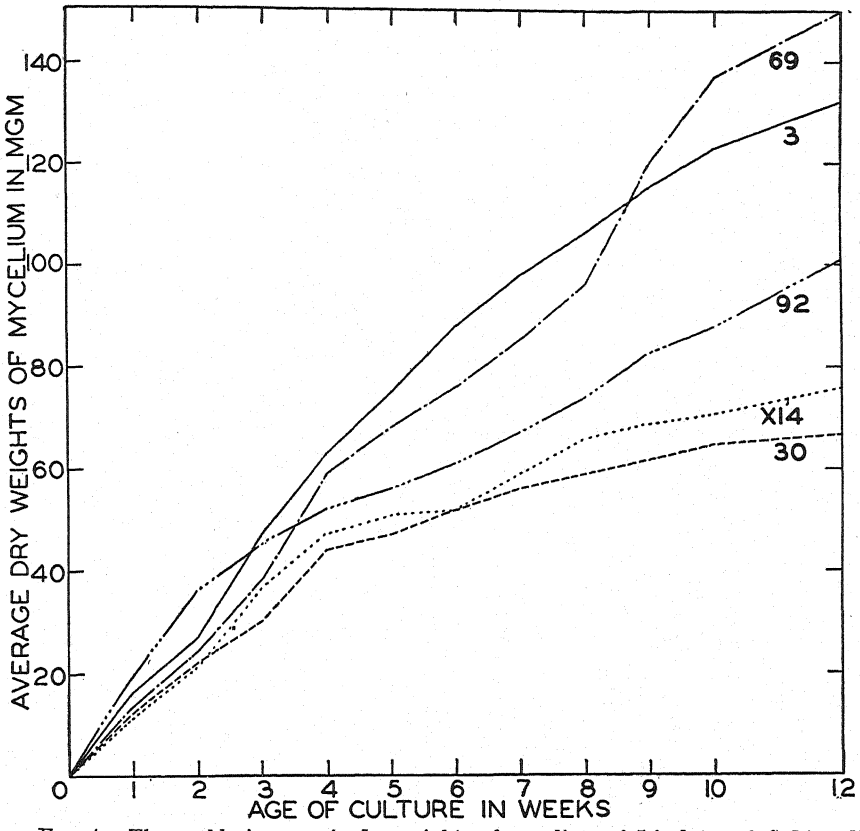


FIG. 4. The weekly increase in dry weights of mycelium of 5 isolates of *C. hiemalis* grown on a liquid medium. Average of 2 series.

The variation in acid production was considerable (Fig. 5). Change in pH was about proportional to the amount of acid produced. The original medium was plus 11.2 Fuller's scale. Isolate 92 changed it in 12 weeks to plus 13.2 and isolate 3 to plus 17.4, other isolates being intermediate in acid production.

The filtrates from the 6-weeks-old cultures of one series were sterilized and reseeded, one of the duplicate filtrates with isolate 30 and the other with isolate 69. The results taken at the end of the 12-week period clearly showed that with both isolates, 30 and 69, the mycelial dry weights of the second seeding were inversely proportional to those of the first seeding (Table 2). This would indicate that the cause for the variation in the amount of growth from the second seeding was the difference in the amount

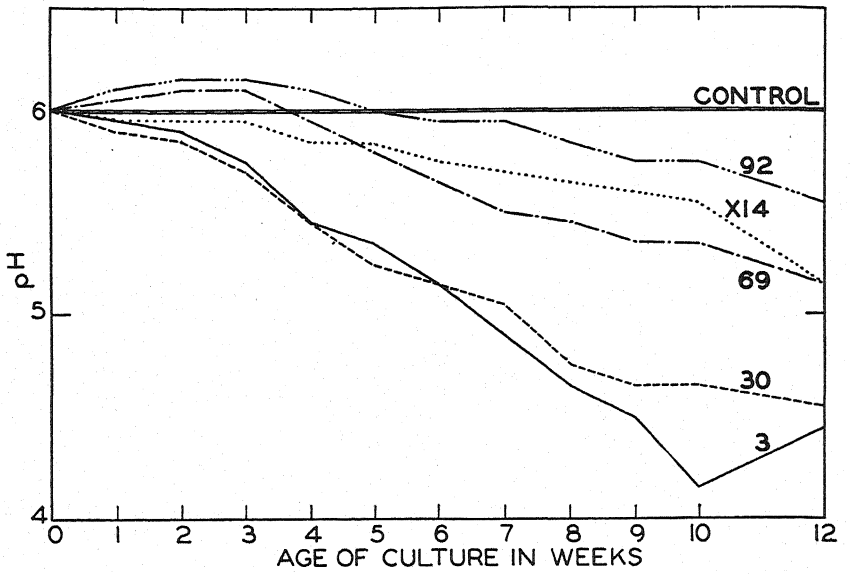


FIG. 5. The change in pH of a liquid medium caused by the growth of 5 isolates of *C. hiemalis*. Average of 2 series.

of nutritive material rather than the presence of staling products. The presence of a toxic material produced in greater amounts by one of the isolates than another was not indicated by these results. Staling products

TABLE 2.—*Results of experiment on growing cultures in a liquid medium partially depleted of nutrients by the growth of other cultures of Coccomyces hiemalis*

Flask[a]	Seeded on 11–23–33		Reseeded on 1–5–34	
	Isolate	Weight of mycelium on 1–5–34	Isolate	Weight of mycelium on 2–17–34
No.	*No.*	*Mgm.*	*No.*	*Mgm.*
1a	43	80	30	14
1b	43	76	69	29
2a	30	47	30	24
2b	30	51	69	34
3a	69	89	30	13
3b	69	97	69	30
4a	92	71	30	16
4b	92	69	69	33
5a	X14	55	30	19
5b	X14	59	69	38

[a] The medium contained in each flask was filtered and autoclaved on 1–5–34 preparatory to reseeding.

may have been present in small amounts or may have been destroyed by autoclaving.

Colonies of the same isolate or of different isolates grown side by side on agar grew equally in all directions and did not show the inhibition of growth in the direction of neighboring colonies so apparent with some fungi. An increase in the number of colonies per plate reduced their size after about 4 weeks. Two colonies of a certain isolate on one agar plate had diameters after 6 weeks twice as great as those of the same isolate on another plate where 12 colonies were growing.

Temperature Relations

The standard agar with 1 per cent malt extract was used in this study. After seeding, the plate cultures were put at 20° for 7 days, when the colonies were measured by taking the average of the longest and shortest diameters. The plates were then placed in 4 replications at 4, 8, 12, 16, 20, 24, 28, and 32°. After 6 weeks from the time of the first measurement, the colonies were again measured and the difference between the first and the second measurements was taken as the amount of growth at the stated temperatures. In preliminary series, measurements taken every week showed the isolates to have about the same relative growth rate from week to week.

The isolates gave similar growth curves, in that their maxima lay between 20° and 24° with a sharp falling off at higher temperatures, no growth taking place at 32°, and a gradual decline in growth at lower temperatures, a small amount occurring at 4°. The relative amounts of growth varied

TABLE 3.—*Effect of temperature on the diameter of colonies of 13 isolates of Coccomyces hiemalis grown on the standard agar with 1 per cent malt extract*

Isolate number	Diameter[a] of colony in mm. after 6 weeks at the stated temperatures							
	4	8	12	16	20	24	28	32
1	3.9	5.3	8.3	12.5	14.4	14.4	9.2	0
3	3.6	6.1	9.5	12.8	17.4	15.6	5.8	0
24	2.9	3.2	4.0	5.8	8.8	10.1	5.9	0
27	2.6	4.1	7.0	8.9	10.4	11.2	6.6	0
29	2.6	3.8	5.6	8.5	10.6	11.6	4.7	0
30	3.8	5.7	8.2	10.3	11.4	11.4	5.2	0
66	3.3	5.6	6.7	8.3	9.8	8.0	5.5	0
69	2.9	5.0	8.6	12.7	16.9	16.3	6.0	0
77	3.3	4.8	8.7	10.4	10.4	10.8	6.4	0
83	2.2	4.1	6.6	7.7	9.2	9.3	6.7	0
X14	3.2	4.8	6.0	8.0	9.2	8.8	7.3	0
91	4.3	5.8	8.5	10.4	15.2	14.0	10.2	0
92	2.0	3.7	5.0	8.0	13.7	14.0	6.3	0

[a] Each diameter is the average of 8 readings taken in 2 series.

considerably between the isolates (Table 3). Isolates 1, 3, 69, and 91 produced colonies at the 3 temperatures, 16, 20, and 24°, with diameters from 51 to 89 per cent greater than the diameters of colonies produced by isolates 24, 66, 83, and X14.

Light Relations

A preliminary survey of the effect of ultraviolet radiation on the sporulation of several isolates of *Coccomyces hiemalis* in culture is reported here.

The action of ultraviolet radiation on fungi has been studied more than that of any other portion of the spectrum, and it is to these rays that fungi are apparently most susceptible. Several investigators have reported 2 effects of ultraviolet radiation on fungi: inhibition or lethal action, and stimulation of fruiting body formation. The former action is reported by Johnson (20) for several fungi showing a suppression of growth from, and adaptive reactions against, the injurious action of ultraviolet radiation. Stevens (45) found a stimulation of the production of fruiting bodies in *Glomerella cingulata* (Atkin.) S. & S. when cultures were exposed to the full range of ultraviolet light for a period as short as 4 seconds. An increase in the sporulation of *Macrosporium tomato* and of *Fusarium cepae* was reported by Ramsey and Bailey (34) to be induced by ultraviolet radiation.

Malt-extract-agar plates, seeded over the entire surface with concentrated spore suspensions, were incubated at 20° for 6 days. The plates were then exposed to the full range of ultraviolet light from a quartz mercury-vapor lamp at a distance of 66 cm. for periods from ½ to 30 minutes. Since no filters were used, the light included some of the visible and infrared range as well as the ultraviolet. Cultures exposed for 2 weeks to diffuse daylight showed no difference in color or spore production from those kept in total darkness. The heat effect of the lamp at 66 cm. was considered negligible.

The effect of the infrared range on cultures of *Coccomyces hiemalis* was not studied. No report of a positive action of this range of wave length on fungi was found in the literature. It is inferred, therefore, that the effect of irradiation reported here was due to the ultraviolet range of radiation.

Four to six days after irradiation, a disc of the agar plate culture was cut out with a large cork borer and placed in a standard amount of water. The spores were thoroughly scraped off into suspension and, after suitable dilution, the number of spores per low power field in 6–8 drops on a slide was counted. Only one field in the center of each drop was used. This was repeated with a second disc of the culture. The average number of spores per low power field was taken as the relative value of sporulation.

Four isolates were used in each of the 3 series made. Isolates 83, 91, 101,

and X14 were repeated in the third series. The results in table 4 give evidence that treatments between ½ and 5 minutes stimulated the cultures to greater sporulation and that longer treatments showed a more or less decided inhibitory effect. Isolates 6, 91, and 101 were stimulated by the shorter period of treatment, whereas cultures 3, 27, 69, 83, and X14 were stimulated only slightly. Treatments of only 3 minutes or more inhibited sporulation of isolate 83, while it took treatments of 10 minutes or more to reduce the sporulation of isolate 101. Treatments of 10, 20, and 30 minutes collapsed the aerial mycelium of all isolates.

TABLE 4.—*Effect of ultraviolet radiation on the sporulation of 8 isolates of Coccomyces hiemalis on 2.5 per cent malt-extract-agar plates*

Isolate number	Average number of conidia per low power field							
	Untreated	Treated with ultraviolet radiation for stated number of minutes						
		½	1	3	5	10	20	30
83[a]	84	109	120	51	43	8	8	9
91[a]	10	81	139	83	68	77	49	47
101[a]	16	64	134	202	361	5	6	3
X14[a]	279	443	244	116	113	87	92	72
3	23	58	.....	43	47	25	5	2
6	102	262	274	204	75	62	68	51
27	129	51	174	224	47	31	17	17
69	21	52	40	35	30	8	16	.....

[a] The results represent the averages of 2 series.

Source of Nitrogen

The sources of nitrogen were used in 0.4 per cent concentration in combination with the basic medium:

KH_2PO_4 ..... 0.4 per cent
$MgSO_4$ ..... 0.05 per cent
Dextrose ..... 2.0 per cent
pH 5.5

The following sources were tested in both the liquid and agar basic medium: peptone, asparagin, aspartic acid, glutamic acid, glycine, ammonium nitrate, potassium nitrate, calcium nitrate, potassium nitrite and urea. In addition, ammonium sulfate, ammonium lactate, and ammonium tartrate were tested in the agar medium.

Cultures were grown at 20° for 6 weeks. Seven isolates, tested in duplicate in 3 series, using 50 cc. of the liquid medium in 250 cc. Erlenmeyer flasks, gave good growth (dry weights of mycelium from 40 to 95 mg.) with peptone. On none of the other sources was good growth obtained. Of

these, glutamic acid gave the best (7–14 mg.). Urea was rather toxic and potassium nitrite prevented germination. With all the sources of nitrogen except peptone, urea, and potassium nitrite, the spores germinated and grew for 1–2 weeks, after which very little, if any, growth took place. The dry weights of mycelium in these cases varied from 2–6 mg. On the basic medium, used alone as a control, germination took place and dry weights of mycelium from 0–2.5 mg. were obtained. Acetamide and alanine used in one series did not support growth of 5 isolates.

The effect of the various sources of nitrogen on the growth of 18 isolates was tested in several series in the basic medium made solid by the addition of 1.7 per cent agar. The agar was not washed. Potassium nitrite inhibited germination of the spores used to seed the plate. Ammonium lactate and urea allowed little or no growth. On all of the other sources the diameters of the colonies produced by any one isolate were very comparable. Even on the basic medium used alone the colony diameters approached those of colonies grown on the peptone medium. Only on the peptone medium, however, did the isolates produce their characteristic colonies. On all the other sources of nitrogen the colonies were thin, with diffuse mycelial growth and no characteristic piling up of the colony above the agar surface.

On the peptone agar and on malt-extract agar, certain isolates produced a pigment that blackened the medium. This black pigment, produced only on peptone or malt extract, may indicate the production of tyrosinase by the fungus, giving a melanin pigment by the oxidation of tyrosin. With any of the other sources of nitrogen, none of the isolates blackened the substrate, nor did they develop their characteristic colors. Some of the isolates produced light shades of pink, yellow or brown on the poor sources of nitrogen. Colonies grown on ammonium lactate, potassium nitrate, and control agars were colorless.

There was little difference between the various isolates tested in regard to their nitrogen requirements, but they showed the usual variation in their relative amounts of growth.

In this study, malt extract used as a source of nitrogen gave results similar to those of peptone.

In preliminary tests, it was found that the addition of small amounts of malt extract to the media, containing peptone or an amino acid, increased the dry weights of mycelium markedly. To test this effect further, 5 isolates were grown in the basic liquid medium, with and without the addition of 0.04 per cent malt extract, and containing peptone, glumatic acid, aspartic acid, glycine, ammonium nitrate, potassium nitrate, or calcium nitrate.

The results of 2 series showed that the presence of the malt extract increased the availability of peptone, glumatic acid, and aspartic acid. In

the case of each of the 5 isolates, the increase in dry weights of mycelium produced on these 3 nitrogen sources by the addition of the malt extract was more, by at least 90 per cent, than could be accounted for by the growth that took place on the basic medium plus 0.04 per cent malt extract alone. In fact, 2 isolates gave more growth on these 2 amino acids than on peptone.

In the case of glycine, ammonium nitrate, potassium nitrate and calcium nitrate, malt extract did not, apparently, make them more available, for the dry weights of mycelium were about the same as those produced by the 5 isolates on the basic medium plus 0.04 per cent malt extract, used as control.

The effect of the addition of 1 per cent yeast extract, 1 per cent malt extract, and 10 per cent sour cherry leaf extract to the standard liquid medium was tested in duplicate, using isolates 30, 91 and X14. The leaf extract was made by steeping 100 grams of Montmorency cherry leaves in a liter of distilled water at 60° for 5 hours. The results show that all 3 of the extracts just about doubled the dry weights of the mycelium in 6 weeks, the leaf extract being about $\frac{2}{3}$ as effective as the other 2 extracts.

The effect of a small amount of malt extract in making some of the amino acids more available is striking. The assumption is that the malt extract contains growth-promoting substances in the category of hormones or vitamins, or rare elements, or some unknown accessory growth factor. Spores will germinate and growth will continue for the first week or two in the absence of the growth promotor, but its presence is apparently necessary for any considerable growth. The spores apparently contain enough of this substance to allow for the initial growth in a medium lacking the growth promotor.

The Parke, Davis and Company bacteriologic peptone, used in this investigation, may contain an amount of the growth promotor, insufficient for best growth of the fungus, for the addition of malt extract, yeast extract, or leaf extract to the peptone medium greatly increases the dry weights of mycelium obtained. The presence of a small amount of a growth stimulator in this peptone may explain why it supports growth where amino acids do not. If this is true, then peptone, without this growth stimulator, might be as poor a source of nitrogen as the amino acids used. The fact that the glutamic acid used in this study was of the "practical" grade, *i.e.*, rather impure, may explain why this amino acid allowed more growth than aspartic acid or glycine which were of C.P. grade.

Source of Carbon

Sixteen isolates were tested in several series for variability in their physiological requirements in relation to the sources of carbon on solid and liquid media. The basic medium consisted of:

Peptone	0.4 per cent	KH_2PO_4	0.4 per cent
$MgSO_4$	0.05 per cent	pH 5.5	

The sources of carbon, added in 2 per cent concentration, were sodium citrate, glycerine, arabinose, l-xylose, dextrose, galactose, levulose, lactose, maltose, sucrose, mannite, salacin, raffinose, inulin, dextrin.

There were no consistent differences between the isolates in regard to source of carbon utilization, all isolates tested showing nearly the same growth relation on any one source of carbon. The only consistent variability noted was in the relative amounts of growth produced by the different isolates.

The sources of carbon are ranked, according to the amount of growth they supported with the various isolates on the liquid and the solid medium, in classes, as follows: good growth on dextrin, dextrose, galactose, lactose, levulose, maltose, mannite, raffinose, and sucrose; fair growth on arabinose and inulin; poor growth on glycerine and salacin. Sodium citrate and l-xylose produced no growth and prevented spore germination.

The results with 3 isolates in one liquid medium series are given in table 5. Isolate 69 is representative of the faster growing isolates and X14 of the slower growing ones.

When, instead of being autoclaved separately, levulose was sterilized with the liquid medium, very poor growth resulted. This effect was not observed when levulose was sterilized with the agar medium or added to the liquid medium after autoclaving.

With most of the sources of carbon the growth of the isolates reduced the pH of the liquid medium considerably. However, with glycerine and especially with inulin the isolates raised the pH of the medium and also blackened it. This may indicate that with these poorer sources of carbon some of the peptone was broken down and utilized as a source of carbon, liberating ammonia and reducing the hydrogen-ion concentration.

MORPHOLOGICAL STUDIES

Considerable variation was found in the length of conidia between some of the isolates of *Coccomyces hiemalis*. Conidia were obtained from 3-week-old Lima-bean-agar cultures and were measured under the high-power magnification, $\times 467$, of the microscope, equipped with a camera lucida. By placing a stage micrometer under the microscope, a scale was drawn on a piece of paper. By replacing the stage micrometer with the conidia, their lengths in microns were obtained by superimposing the scale upon their image. The use of a mechanical stage guarded against replicate measurements of any spore. The measurements were made to the nearest 5-micron interval. The conidia were mounted in lacto-phenol. A small amount of fast green or safranin dissolved in the mounting fluid proved helpful.

TABLE 5.—*The effect of various sources of carbon on the dry weights of mycelium and final pH of cultures grown for 6 weeks at 20° in liquid media with an original pH of 5.5*

Sources of carbon	Isolate 69		Isolate 83		Isolate X14	
	Average dry weights of mycelium	Final pH	Average dry weights of mycelium	Final pH	Average dry weights of mycelium	Final pH
	Mgm.		*Mgm.*		*Mgm.*	
Control	12	6.1	6	5.9	5	5.8
Arabinose	45	5.5	29	5.9	12	5.4
Dextrose	57	5.4	47	5.6	27	4.1
Dextrin	59	5.3	44	5.6	38	4.5
Galactose	46	4.7	35	5.4	29	4.5
Glycerine	29	6.3	25	6.1	14	6.0
Inulin	36	6.6	32	6.3	15	6.3
Lactose	46	4.5	40	5.5	32	4.6
Levulose	56	5.3	43	5.4	29	4.5
Maltose	53	5.3	40	5.3	33	4.2
Mannite	57	4.9	41	5.3	28	4.3
Raffinose	46	5.0	38	5.4	33	3.9
Salicin	21	4.7	20	5.5	22	4.5
Sodium citrate	0	5.2	0	5.3	0	5.2
Sucrose	50	4.9	50	5.3	23	5.1
l-Xylose	0	5.5	0	5.5	0	5.6

Many of the spores were more or less curved. These were measured by taking the length of an imaginary median line drawn through the conidium from end to end. This was done by comparing the curved spore with one of several calibrated lines, drawn on a paper, having curves corresponding to those of the spores.

Length of Conidia in Culture

The distribution in length of 500 spores each of 6 isolates in culture is shown in figure 6, A. These isolates represent the extreme of variation in conidial length as found in pure culture. The curves for isolates 69 and 91 with modes at 50 and 60 microns, respectively, are representative of the curves for 5 other isolates studied.

The average length of spore for each isolate corresponds closely with the modal length of its curve. The average spore length in microns for these 6 isolates is 45.0 for No. 30, 49.0 for No. 3, 51.2 for No. 91, 55.8 for No. 69, 67.5 for No. X14, and 78.9 for No. 83. Ninety-three per cent of the spores of isolate 30 are shorter than 60 microns and 94 per cent of isolate 83 are longer.

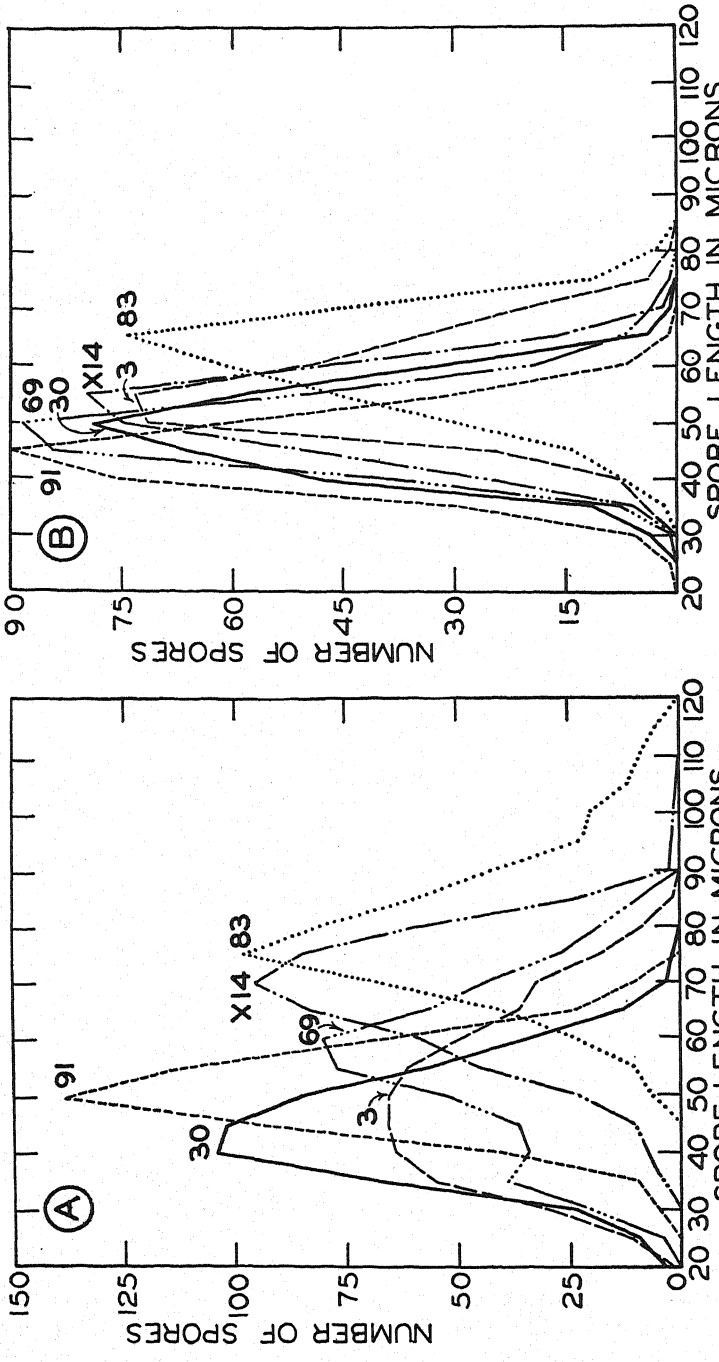


FIG. 6. The distribution in length of conidia of 6 isolates of *C. hiemalis* using (A) 500 spores of each isolate from Lima-bean-agar cultures, and (B) 300 spores of each isolate from Montmorency sour cherry leaves.

Length of Conidia on Several Hosts

Conidia of these several isolates when produced on Montmorency cherry (*Prunus cerasus* L.) leaves were found to have lengths different from those found in culture. Samples of spores to be measured were obtained from comparable leaves that were inoculated on the same date and whose lesions were 2 weeks old, 300 conidia of each isolate being measured.

The distribution of spore length for the same 6 isolates when conidia were obtained from leaves is represented in figure 6, B. The isolates varied much less in length of conidia when the latter were obtained from Montmorency cherry, and these conidia were in general shorter and more uniform in length than they were from culture. The average spore length in microns for the 6 isolates is 44.9 for No. 91, 48.8 for No. 30, 48.9 for No. 69, 51.8 for No. X14, 55.6 for No. 3, and 60.5 for No. 83.

Higgins (18) gave the range in length of conidia of *Coccomyces hiemalis* to be from 45–65 microns. The writer found the conidia of isolate 91 from sour cherry leaves to be from 25–65 microns in length and the length of conidia of isolate 83 from the same source to be from 35–80 microns. The range in length of conidia from culture is much greater (Fig. 6, A).

Little variation was found between the isolates in the number of septa per spore. The per cent of conidia from the host having one septum varied from 84 to 100. As many as 16 per cent of them had 2 septa, and less than 1 per cent had no septa. Lima bean agar cultures, on the other hand, differed in having 3 to 8 per cent of non-septate, 3 to 10 per cent of 2-septate, and an occasional 3-septate conidium.

A limited study of the width of conidia under oil-immersion objective indicated but slight variation in this regard between the isolates. The maximum diameter of conidia from culture or host varied from 1.5 to 3.4 microns with an average of 2.0–2.7 for the different isolates. Higgins reported the width to be from 2.5–4 microns.

The effect of other species of Prunus on the size of conidia was tested with 4 isolates, 200–300 spores being measured in each case. The average length of conidia produced on *P. pennsylvanica* L. was for isolate 30, 48.4 microns; for isolate 69, 45 microns; for isolate 83, 58.3 microns; for isolate X14, 54 microns. These averages compare rather closely with those obtained for spores produced on *P. cerasus*. On *P. mahaleb* L. the average length of conidium with X14 was 55.4 microns and on *P. cerasifera* Ehrh. was 44.7 microns. Sufficient spores for measurements of other isolates were not obtained from *P. cerasifera* due to the difficulty of finding sporulating lesions.

The effect of different environments on the length of spores produced on Montmorency was studied, using isolate X14. Two control chambers devised by Keitt for growing plants under controlled temperature, humidity,

and illumination were used in this study. One of these chambers was run at a temperature of 24° with a full complement of artificial light and the other at 14° with 1/3 of this light intensity. The day and night periods were 12 hours each. The humidity in both chambers varied from 75 to 90 per cent of saturation. The experimental trees were put in the chambers immediately after the moist treatment following inoculation with isolate X14. Leaves were sampled for spores 8 to 12 days after the lesions appeared and from each chamber 250 spores were measured. This was repeated with trees inoculated several days later, giving over 500 measurements from each chamber.

The spores from the low-temperature chamber varied in length from 40 to 80 microns, averaging 59.1, and those from the high-temperature chamber varied from 30 to 65 microns, averaging 47.9 microns. Further work to determine whether this variation was due to difference in temperature or in light was not feasible.

Size of Ascospores

Measurements of ascospores from sour cherry leaves obtained from different localities in Wisconsin in 1916–1917, and in 1932, showed little variation in average spore length or average width between different samples.[5]

Ascospores were shot onto plates of 1.7 per cent agar and measured as described for conidia. Their lengths varied from 25–30 to 55–65 microns, averaging between 42 and 49 microns in the various samples. The ascospores had an average width of 3.0 to 4.2 microns. Higgins' measurements for ascospores were 33–50 × 3.5–4.5 microns.

INFECTION STUDIES

Higgins (18) and Keitt (24) reported *Coccomyces hiemalis* to be pathogenic on *Prunus avium* L., *P. cerasus,* and *P. pennsylvanica;* and the latter author showed, in addition, that *P. mahaleb* was susceptible also. Keitt found that the various Prunus species exhibit a gradation of susceptibility toward *C. hiemalis* from complete susceptibility, through degrees of resistance, to immunity. He showed that this fungus sometimes infected the Damson plum (*P. insititia* L.) after a prolonged incubation period, and in addition produced aberrant lesions under certain conditions on other species and varieties of plums. These facts might suggest pathogenic variability between isolates of *C. hiemalis.*

A number of fungus pathogens in addition to the rust and smut fungi have been found to contain physiologic forms. Some of the more important will be mentioned here. Neger (32), Marchal (28), Salmon (37), and Reed (35) showed that several of the powdery mildews exhibit physi-

[5] The writer is indebted to Dr. G. W. Keitt for part of these data.

ologic forms. Eberhardt (13) described 2 pathogenically specialized races of *Albugo candida* (Pers.) Gray. Five specialized races were found in *Claviceps purpurea* (Fr.) Tul. by Stäger (40). Müller (31) made a study of *Rhytisma acerinum* (Pers.) Fr. on various maples and concluded that it consists of several specialized races. Gilbert (16) found biologic forms in *Plowrightia morbosa* (Schw.) Sacc. Gaumann (15) reported a high degree of host specialization in *Peronospora parasitica* (Pers.) de Bary. Wormald (49) found physiologic forms in *Sclerotinia cinerea* (Bon.) Wor. Palmiter (33) reported strains of different pathogenic capabilities in *Venturia inaequalis* (Cke.) Aderh.

Methods and Materials

Two-week-old Lima-bean-agar cultures and 2- to 3-week-old lesions on Montmorency sour cherry leaves were used as sources of spores for inoculations. Spores obtained from lesions were more vigorous in germination on 1.7 per cent agar in water than spores from culture. Comparative tests of the infection produced on Montmorency by spores from infected leaves and from culture, when the spore suspensions were standardized to 100 spores per low power field, showed that spores from the leaves gave as much as 50 per cent more infection than spores obtained from Lima-bean-agar cultures.

The spore suspensions of the various isolates being compared were made up to contain about 100 spores per low power field. The suspensions were sprayed on the lower surface of the leaves with an atomizer until the water ran off.

Two- and three-year-old potted trees, limited to 2–4 shoots each, were used in these studies. For any one series, trees with uniform shoot growth were selected. The inoculated trees were immediately placed in the moist chamber, devised by Keitt (25), at a temperature of 20°, where they remained for 48 hours. Subsequent to the moist treatment, the trees were kept in a greenhouse. About 3 weeks after inoculation, data were taken by counting the number of lesions in the maximally infected square inch of each leaf.

Infection Studies on Sweet and Sour Cherry

Using spores from culture and from the host, 17 isolates were tested comparatively for the amount of infection produced on Montmorency sour cherry during the spring of 1932 and of 1933. Each isolate was used in at least 3 series. Isolates 1, 52 and X25 were tested with spores from culture only. These gave either no infection or such sparse infection that sufficient inocula for reinoculations could not be obtained from the leaves. It is interesting to note that these isolates produced relatively few spores and all of them are classed according to color as flesh pink. The other

isolates used gave fair to good infection on Montmorency in all tests. The average infection per shoot varied in amount from 25 to over 150 lesions per maximally infected square inch, when spores from leaf or culture were standardized to 100 per low power field.

Data were taken 3–4 weeks after inoculation on the per cent of necrotic lesions. These lesions were 1–2 mm. in diameter, brown, and in the process of being corked out to produce "shot-holes." Greenhouse temperatures under 16° were unfavorable and over 20° favorable for the appearance of necrotic lesions. Under the conditions of the experiments, the following isolates produced, after 3–4 weeks, from 25–80 per cent necrotic lesions: 21, 27, 30, 49, 66, X2, and X12. Twelve other isolates gave from 0–30 per cent necrotic lesions: 3, 6, 17, 24, 29, 69, 76, 77, 83, 91, 92, and X14.

Prunus mahaleb gave results in a few trials similar to those of *P. cerasus,* except that infection was much less heavy and necrotic lesions did not develop.

Spores from cultures of each of 21 isolates, used on Governor Wood sweet cherry (*Prunus avium*), gave some infection with the exception of isolates 1, 11, 52, 87, and X25, which gave none. With all of the other isolates infection varied between different tests from poor to good, the variation being inconsistent.

In the 4 series, using Governor Wood, data were taken on the type of lesions produced by each isolate. It was found that some gave lesions, practically all of which had acervuli, and others produced relatively few sporulating lesions. The isolates producing sporulating lesions were 17, 20, 24, 30, 69, 77, X12, X14, and X27. The following gave the aberrant type of lesion: 3, 6, 21, 27, 66, 83, and X53. It was noticed that the lesions without acervuli were rather quickly corked out, even when very small. This corking out usually took place within 4–5 days after the appearance of the lesions and it was not usually attended by necrosis.

Prunus pennsylvanica was also used to test the pathogenicity of several isolates. This host proved unsatisfactory, due to much variation between the seedling plants. Isolates produced sporulating lesions on one plant and aberrant ones on another, both in the same series and in different series. Different isolates when inoculated on one plant gave essentially the same type of lesion. The type of lesions varied between plants from necrotic to non-necrotic and from aberrant to sporulating. Young leaves seemed to be more favorable for the production of lesions with acervuli.

Infection Studies on Other Species of Prunus

Prunus domestica L., *P. munsoniana* Wight & Hedr., *P. insititia* L., and *P. cerasifera* proved unsatisfactory as experimental material, for sparse and erratic infection resulted with the several isolates tried, the lesions

usually being aberrant. The results of one inoculation experiment did not conform with those of the next, due in part perhaps to the difference in age and condition of the leaves.

DISCUSSION

The isolates of *Coccomyces hiemalis* included in this study have been shown to vary to some extent in certain physiological reactions and in length of conidia. These variations are probably no greater in extent than would ordinarily be expected or found in single spore isolates of any fungus. Similar or greater variation has been reported by Hansen and Smith (17) for *Botrytis cinerea* Pers., by Matsumoto (29) for Rhizoctonia, by Stevens (44) for Helminthosporium, by Shear and Wood (39) for Glomerella, by Brown (4) for Fusarium, and by Mohendra and Mitra (30) for *Sphaeropsis malorum* Pk. As a result of his extensive studies of many isolates of *Botrytis cinerea,* Brierley (3) pointed out the great variation possible within a single fungus species.

The theories regarding the origin of variants in this fungus would probably include hybridization, which has been shown to be a cause of variation in some rusts, notably by Craigie (8), Stakman *et al.* (43), and Johnson *et al.* (21), and in smuts by Stakman *et al.* (42), Holton (19), and Rodenhiser (36), as well as in the ascomycete Neurospora by Dodge (9) and in Sclerotinia by Drayton (12). *Coccomyces hiemalis* has not been shown to contain self-sterile and inter-fertile thalli. However, Backus (1) in his paper on the development of the ascus in *C. hiemalis* refers to some of his unpublished data which may demonstrate the existence of heterothallism in this fungus.

Discontinuous variation occurred in many of the isolates of *Coccomyces hiemalis* in culture, usually as pie-shape sectors. Variation was manifested in colony color and contour, and in growth rate. Some of the isolates were very unstable for several generations after isolation from single ascospores. The unstable isolates were not used in these studies. Brierley (3) states that some isolates of *Botrytis cinerea* are extremely unstable in culture while others are distinguished by rarely producing discontinuous variants. "The degree of discontinuous variability, ranging from forms which seem entirely stable to forms which are ever sporting, is characteristic of the individual isolate." One of his albino isolates was stable for 13 years.

The isolates of *Coccomyces hiemalis* were found to be unsuited to the study of discontinuous variation or sectoring in culture due to the very slow growth and small size of the colonies. Growth from single spores was especially slow.

Small amounts of malt extract, yeast extract, and cherry leaf extract have been found to stimulate the growth of this fungus. The growth-pro-

moting action of these substances may be due to one or more rare elements, amino acids, peptide linkages, a hormone or vitamin, or some unknown accessory growth factor. The growth of some lower forms of life, including yeasts and other fungi, have been shown to be greatly stimulated by the addition to the nutrient medium of growth stimulators. The accessory growth factor has been variously described as "bios," "auximones," glutathione, and pantothenic acid by different workers. Buchanan and Fulmer (5) gave an excellent review of the subject of accessory growth factors. Tanner (46) discussed the "bios question" and Williams and Honn (48) the rôle of "nutrilites" in the nutrition of molds and other fungi.

Conidia of *Coccomyces hiemalis* apparently contain a small amount of a growth promotor, since in a medium containing inorganic salts and dextrose, growth proceeded for several days and then ceased. The results of the experiments indicate that some substances, containing complex protein material, greatly stimulate the growth of cultures of this fungus.

Although some isolates differed in their pathogenicity on the leaf, the results of the inoculation experiments do not indicate the existence of physiologic forms. There are many factors that play into the host-parasite relation and that modify the amount of infection and type of lesion obtained. Some of these factors are temperature, light, the age and vigor of the leaf, variation between individual host plants, and the vigor of spores used for inoculum.

SUMMARY

A number of single ascospore isolates of *Coccomyces hiemalis,* obtained from Michigan, New York, New Jersey, Pennsylvania, Oregon, and Wisconsin, were studied from the standpoint of variability in culture and on several hosts. These isolates were found to vary:

A. In colony characters, including color, contour, growth rate, and ability to blacken the substrate.

B. In spore production, some isolates producing several times more conidia than others on Lima-bean agar. Isolates were found to produce microconidia on the host, but only a few produced them in culture under the conditions studied.

C. In growth rate and acid production in a liquid medium.

D. In reaction to ultraviolet radiation, short periods, in general, stimulating sporulation and longer periods inhibiting sporulation as well as collapsing aerial mycelium.

E. In the length of conidia produced in culture and on the host. A combination of low temperature and low light intensity resulted in longer conidia from the host than were developed with a higher temperature and more light.

F. In the amount of infection and type of lesion produced on sweet and sour cherry leaves.

Conidia from cultures of several isolates were found to germinate over a range of pH from 2.9 to 8.5 with a maximum for germ-tube length at about pH 5.4.

Colonies an agar grew and produced conidia at temperatures from 4–28° C., no growth or sporulation occurring at 32°. The optimum for growth lay between 20° and 24° for all isolates, yet at these temperatures certain isolates grew almost twice as rapidly as others. The optimal temperature for spore production varied with the different isolates, some producing more at 12° and others more at 16–20° C.

Of a number of organic and inorganic sources of nitrogen tested in a liquid and a solid medium, peptone alone was found to allow good growth of the isolates. In the presence of a small amount of liquid malt extract, glutamic acid and aspartic acid also supported good growth. Malt extract, yeast extract, and cherry leaf extract acted as growth stimulators.

Many mono- and disaccharides as well as dextrin, inulin, mannite, raffinose, and arabinose supported growth in a medium, liquid or solid, containing peptone and nutrient salts. Glycerine was a poor source of carbon and sodium citrate and l-xylose were toxic.

The isolates from any one locality varied as much as those from widely different localities.

A few isolates that sporulated sparingly in culture, or that produced feebly germinating conidia, were found to be either non-pathogenic or weak pathogens on sweet and sour cherry. The existence of physiologic forms of the fungus was not indicated.

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INCUBATION PERIOD OF PEA MOSAIC IN THE APHID, MACROSIPHUM PISI[1]

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INTRODUCTION

Although aphids are known to spread a considerable number of plant-virus diseases, little attention has been given to the manner in which they carry and transmit the viruses causing these diseases. For such a study the pea aphid, *Macrosiphum pisi* Kaltenbach, offers many advantages, being a relatively large insect, easy to handle in experiments, and easy to maintain in culture. Moreover, it is the carrier of a pea mosaic that is difficult to transmit by mechanical methods.

In the transmission of several plant viruses by leaf hoppers and thrips it has been demonstrated that an interval, referred to as an incubation period, must elapse after the insects first feed on diseased plants before they are capable of infecting healthy plants. It has also been shown that these vectors retain the virus for considerable periods of time. This has led some investigators to hold the view that viruses may multiply or pass through a stage of development in insect vectors.

The evidence that plant viruses may undergo an incubation period in aphid vectors is very meager. The only instance thus far reported is for the virus of potato leaf roll in the aphid *Myzus persicae* (Sulzer). For several other viruses it has been shown that if an incubation period occurs it is extremely short. It was of particular interest, therefore, to determine whether or not transmission of pea mosaic by the pea aphid involves an incubation period of the virus in the insect. It also seemed of interest to determine whether or not the virus is retained by the insect for a considerable period of time. In cases in which leaf hoppers serve as vectors and in which an incubation period has been demonstrated, transmission is known to occur in each instance through the agency of only a single species of insect. Moreover, mechanical transmission of the diseases is difficult or has not been obtained. It was desirable, therefore, to determine whether or not pea mosaic can be transmitted by other insects than the pea aphid, and to what extent it can be transmitted by mechanical methods. The purpose of this paper is to present the results of experiments undertaken in a study of these points.

REVIEW OF LITERATURE

Taubenhaus (18) appears to have been the first to report the pea aphid as a carrier of a mosaic disease. He found that it would transmit mosaic

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in sweet peas. The pea aphid also has been reported as a carrier of mosaic diseases in peas and clover by Dickson (2), Doolittle and Jones (3), and Böning (1). It has been reported by Zaumeyer (19) as the carrier of mosaic from peas and sweet peas to beans. These studies, however, were not concerned with the mode of transmission by the pea aphid, and no reference was made regarding an incubation period of the virus in the insect.

Smith and Boncquet (15) were apparently the first to report on incubation period of a plant virus in an insect carrier. They found that the virus of curly top of beet had an incubation period of 24 hours in the beet leaf hopper. Once acquired, the virus of curly top of beet was shown by Stahl and Carsner (16) to be retained by the insect throughout its life. Severin (12) found that at high temperatures (100° F.) the minimum incubation period of curly-top virus in the beet leaf hopper might be as short as 4 hours.

An incubation period has been shown by Kunkel (9) for aster yellows in the leaf hopper, *Cicadula sexnotata* Fallen, and by Storey (17) for maize streak in the leaf hopper, *Cicadulina mbila* (Naude). It has also been shown by Samuel *et al.* (11) for spotted wilt of tomato in the thrips, *Frankliniella insularis* (Franklin), and by Linford (10) for yellow spot of pineapple in *Thrips tabaci* Lindeman. The papers of Elze (6) and Smith (13, 14) indicate that the potato leaf-roll virus undergoes an incubation period in the aphid, *Myzus persicae.*

On the other hand, the viruses of certain diseases, such as cucumber mosaic and yellow dwarf of onions, do not require an incubation period in aphids known to serve as vectors. Doolittle and Walker (4) found, in studies on the transmission of cucumber mosaic by the melon aphid, *Aphis gossypii* Glover, that the insects acquired the virus in a period of 5 minutes on a diseased plant and were able to infect healthy plants within an equally brief period of feeding. They lost the ability to infect healthy plants soon after being removed from diseased plants. Hoggan (7) states that, in the transmission of cucumber-mosaic virus to tobacco, the peach aphid, *Myzus persicae,* acquired and transmitted the virus in a total period of not more than 30 minutes, and that it lost its infectivity after feeding for a 2-hour period on a healthy plant. Drake *et al.* (5) found that 50 different species of aphids served as carriers of yellow dwarf of onions. If the virus requires an incubation period in the insects reported as carriers, this period is extremely short. Furthermore, the aphids lost the virus in a short period of time after removal from diseased plants.

MATERIALS AND METHODS

The mosaic disease used in these experiments was obtained from *Vicia faba* L. plants growing in Westchester County, New York. The disease is

referred to in this paper as pea mosaic. In order that material might be available at all times, the disease was maintained continuously in a greenhouse by successive transfers to fresh plants.

Colonies of the pea aphid were reared on healthy *Vicia faba* plants in screened cages. Inoculations were made by transferring aphids that had fed on diseased plants to small healthy plants under screened cages or lantern chimneys. The cages and chimneys used were similar to those described by Kunkel (9).

In all of the experiments equal numbers of exposed and control plants were grown. Except for the differences noted below, the controls were given a treatment similar to that afforded the plants under test. Aphids that had been reared on healthy plants were divided into two lots. One lot was placed on diseased plants and the other was left on healthy plants. Aphids of the lot that had fed on diseased plants were then used to inoculate plants under test, while the control plants were exposed to aphids from the lot that had fed only on healthy plants. At the end of an exposure period the aphids were removed and the plants fumigated with Nicofume before return to a greenhouse.

HOST RANGE AND DESCRIPTION OF THE DISEASE

Pea mosaic was transmitted by means of the pea aphid from *Vicia faba* to garden pea, *Pisum sativum* L., field pea, *P. sativum* var. *arvense* Poir, sweet pea, *Lathyrus odoratus* L., and crimson clover, *Trifolium incarnatum* L. By the same method it was transmitted back from each of these plants to *V. faba*. Attempts to transmit the disease to red clover by means of the pea aphid and by mechanical methods were not successful. Several mosaic diseases have been observed in *V. faba,* peas, and crimson clover. A description of the disease with which this paper is concerned is, therefore, presented here.

Diseased plants of *Vicia faba* are characterized by a conspicuous mosaic pattern that appears in the leaves. This pattern, which takes the form of a spotting rather than of a mottling, is illustrated in figure 1, A. The spots are somewhat irregular in shape and quite variable in size. They tend to appear along the veins and in some plants expand into broad stripes. Symptoms of the disease have occasionally been observed 5 days after inoculation. Usually they appear in from 6 to 14 days. Some stunting has been observed in diseased plants, though this is usually not severe.

When transmitted to the Telephone variety of garden pea, the disease produces a wrinkling of the leaves with some stunting of the plant. Yellowish spots that appear on the leaves later become white and somewhat transparent. Enations, similar to those described by Jensen (8) as resulting from tobacco-mosaic infection in certain species of Nicotiana L., occur

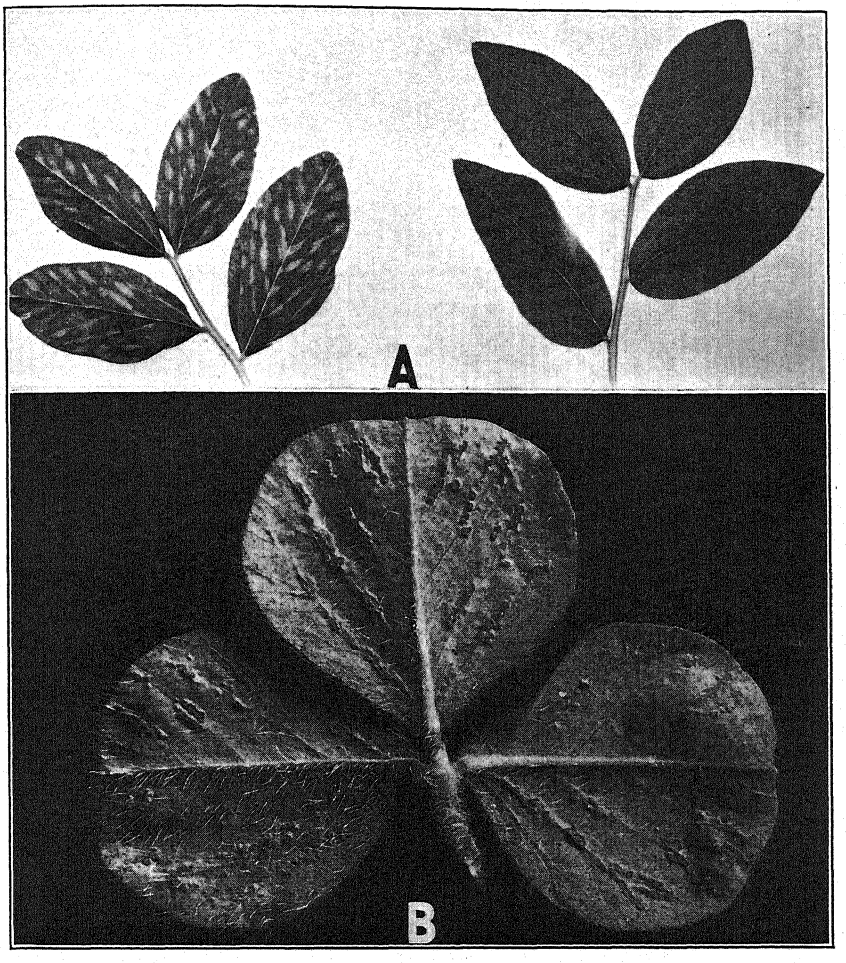


FIG. 1. A. *Vicia faba* leaves infected with pea mosaic from diseased *V. faba* by inoculation with the pea aphid. Left, from diseased plant; right, from healthy plant. B. Under surface of leaf from crimson clover plant showing enations produced by pea mosaic. Enlarged more than twice natural size.

on the under surface of the leaves. An advanced stage of the disease in Telephone pea is shown in figure 2, A.

In diseased sweet peas there is a pronounced wrinkling of the leaves. The mosaic pattern differs from that in the Telephone variety of garden pea in that the spots usually are elongated to such an extent that they might well be described as streaks. A diseased sweet-pea leaf is illustrated in figure 2, B.

Diseased plants of crimson clover are characterized by small yellowish

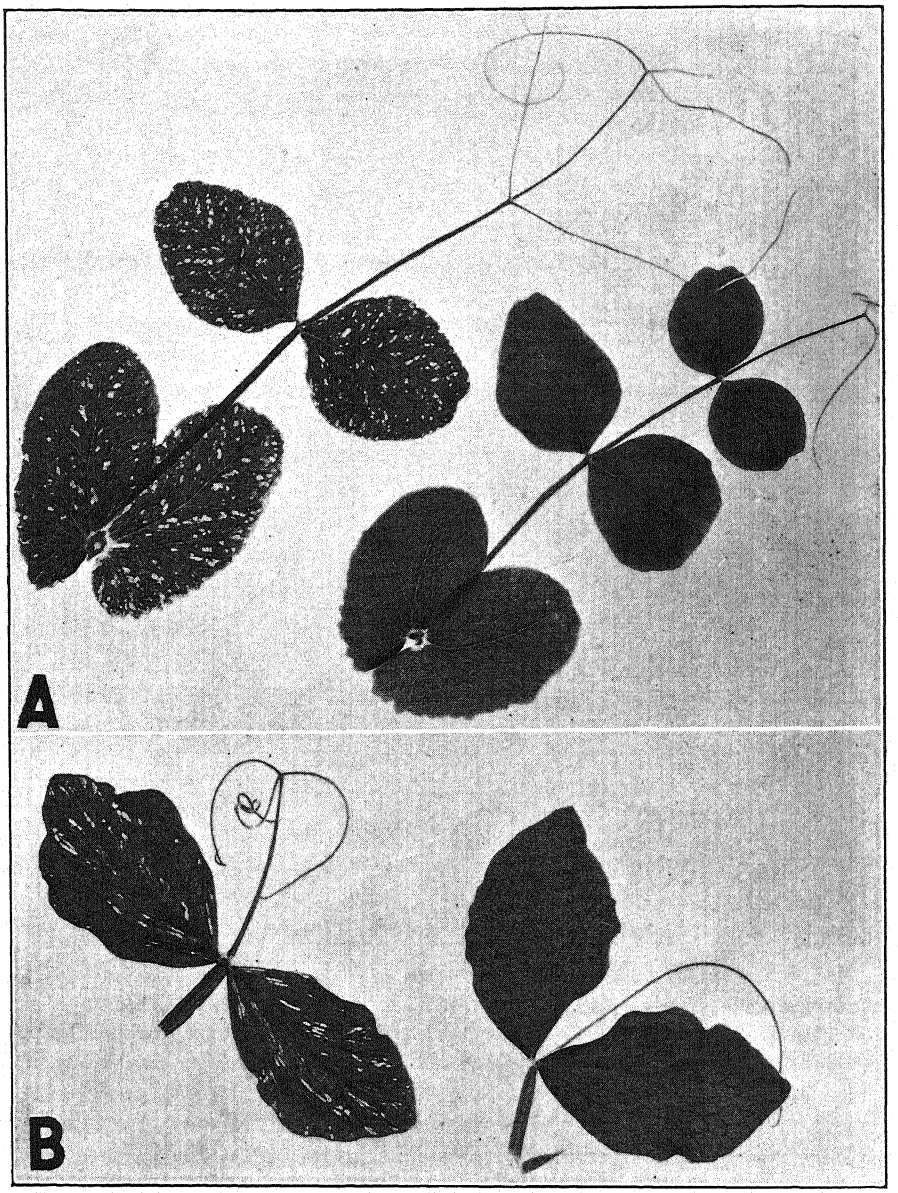


FIG. 2. A. Telephone pea infected with pea mosaic from diseased *Vicia faba* by inoculation with the pea aphid. B. Sweet pea infected with pea mosaic from diseased *Vicia faba* by inoculation with the pea aphid. In both A and B, leaves at left from diseased plant; those at right, from healthy plant.

spots on the upper surface of the leaves and distinct enations, such as are shown in figure 1, B, on the under surface of the leaves.

INCUBATION PERIOD OF THE VIRUS IN THE PEA APHID

A series of experiments was undertaken to determine whether or not transmission of the disease involves an incubation period of the virus in the insect. For this purpose colonies of the pea aphid were transferred from diseased to healthy *Vicia faba* plants. *Vicia faba* was used in all of the experiments for the reason that it is highly susceptible to pea mosaic, grows rapidly, serves as a satisfactory food for the aphids, and facilitates transfer of large colonies because of its broad smooth leaves. In preliminary experiments with colonies, it was found that if an incubation period existed it was less than 48 hours. Therefore, colonies of aphids were allowed to feed on diseased plants for periods of 2 to 12 hours, and were subsequently transferred to successions of healthy plants. The colonies consisted of from 50 to 200 insects in the nymphal stage except in one experiment where adults were used. The use of large numbers of aphids in colonies appeared to be desirable, since all of the insects placed on diseased plants do not acquire the virus even when allowed to feed for a 24-hour period. Moreover, single insects, even though carrying the virus, do not always infect healthy plants during a feeding period of 24 hours.

In one experiment conducted in a greenhouse, 5 colonies of pea aphids were fed for 8 hours on diseased plants and then transferred to a succession of healthy plants. The colonies in each case consisted of 50 adult insects. After removal from the diseased plants, 5 transfers were made at intervals of 4 hours and subsequent transfers at irregular intervals. The temperature was held at approximately 67° F. during the time the insects were on diseased plants and during the first 4 periods on healthy plants. For a short time during the 2nd day, due to bright sunlight, the temperature rose to 76° F. The infections obtained in this experiment are shown in table 1. Four of the colonies were infective. No infection, however, was obtained in any of the healthy plants exposed to the colonies during the first 4 periods. Two of the colonies became infective after an incubation period of not less than 16 nor more than 28 hours, and 2 of them after an incubation period of not less than 20 nor more than 36 hours. The experiment thus demonstrated an incubation period in adult pea aphids.

In a second experiment, similar to the preceding, nymphs were used instead of adults. Five colonies, consisting of 50 large nymphs each, were fed for 12 hours on diseased plants in a greenhouse that was held at a temperature that varied from 64° to 70° F. Twelve transfers were then made to healthy plants at 2-hour intervals, and subsequent transfers at irregular intervals. The infections resulting from these exposures showed that one

TABLE 1.—*Infections obtained with 5 colonies of adult pea aphids when fed for 8 hours on diseased Vicia faba plants and then transferred to a succession of healthy plants*

Period	Length of exposure	Approximate temperature during exposure	Succession of plants				
			Colony 1	Colony 2	Colony 3	Colony 4	Colony 5
1st	4 hrs.	67° F.	−	−	−	−	−
2nd	" "		−	−	−	−	−
3rd	" "		−	−	−	−	−
4th	" "		−	−	−	−	−
5th	" "	70° F.	+	+	−	−	−
6th	8 hrs.		+	+	+	+	−
7th	5 hrs.		+	+	−	+	−
8th	2 hrs.	76° F.	+	+	+	+	−
9th	17 hrs.		+	+	+	+	−
10th	24 hrs.		+	+	+	+	−
11th	" "		+	+	+	+	−
12th	" "		+	+	+	+	−

+ = Plant became infected.
− = Plant remained healthy.

colony became infective after an incubation period of not less than 10 nor more than 24 hours, 2 colonies became infective after an incubation period of not less than 20 nor more than 36 hours, and one colony was infective after a period of not less than 72 nor more than 96 hours. One colony failed to infect any plants on which it was placed. This experiment demonstrated an incubation period in nymphs of the pea aphid.

In one experiment conducted in a greenhouse, the temperature varied from 74° to 76° F. during the time the insects were on diseased plants, and was held above 70° F. during subsequent transfers. Five colonies, each consisting of 100 nymphs 2 to 4 days old, were allowed to feed for 6 hours on diseased plants and then transferred to a succession of healthy plants. Transfers were made at intervals of 2 hours to 9 sets of healthy plants and thereafter at irregular intervals. The infections resulting from the exposure of healthy plants to the colonies of aphids in this experiment are shown in table 2. Plants exposed to 2 of the colonies during the first period became infected. Two colonies, however, became infective only after an incubation period of not less than 6 nor more than 14 hours. For some unknown reason, one colony infected but a single plant. This experiment, therefore, demonstrated an incubation period in 2 colonies, but failed to demonstrate an incubation period in 2 other colonies, because some of the insects were infective within less than 8 hours after first feeding on diseased plants.

TABLE 2.—*Infections obtained with 5 colonies of pea aphids in the nymphal stage when fed for 6 hours on diseased plants and then transferred to a succession of healthy plants*

Period	Length of exposure	Approximate temperature during exposure	Succession of plants				
			Colony 1	Colony 2	Colony 3	Colony 4	Colony 5
1st	2 hrs.	72° F.	+	−	−	+	−
2nd	" "	"	−	−	−	+	−
3rd	" "	70–72° F.	+	−	−	+	−
4th	" "	"	+	+	+	+	−
5th	" "	"	+	−	−	+	−
6th	" "	"	+	+	+	+	−
7th	" "	"	+	+	+	+	−
8th	" "	70–80° F.	+	−	+	+	+
9th	" "	"	+	+	+	+	−
10th	24 hrs.		+	+	+	+	−
11th	" "		+	+	+	+	−
12th	" "		+	+	+	+	−

+ = Plant became infected.
− = Plant remained healthy.

An experiment was, therefore, conducted in which exposure to diseased plants was shortened. Five colonies were allowed to feed for 2 hours on mosaiced plants. The colonies consisted of 200 nymphs each. Transfers to healthy plants were made at intervals of 2 hours. The greenhouse was held at a temperature of approximately 80° F. while the insects were on diseased plants. Due to weather conditions, the temperature varied during the remainder of the experiment from 72° F. to 90° F. In making transfers of such large numbers of aphids at frequent intervals, some loss of individuals occurred. A count made at the 12th transfer showed that the number of insects remaining in the colonies varied from 165 to 190. The infections resulting from exposure of healthy plants to the colonies of aphids in this experiment are shown in table 3. All of the plants exposed to the colonies during the 1st and 2nd periods remained healthy. A plant exposed to one of the colonies during the 3rd period of the series became infected, as did a plant exposed to another colony in the 4th period. One colony became infective during the 7th period, and another during the 9th period. One infection obtained in this experiment showed that at high temperatures (80° F. in this case) some of the insects became infective within a period of not more than 8 hours after they first fed on diseased plants. Even with transfers of 200 insects to each plant, no infections occurred in plants exposed during the 1st and 2nd periods after removal from diseased plants. This showed that even at high temperatures the

TABLE 3.—*Infections obtained with 5 colonies of pea aphids in the nymphal stage when fed for 2 hours on diseased plants and then transferred to a succession of healthy plants*

Period	Length of exposure	Approximate temperature during exposure	Succession of plants				
			Colony 1	Colony 2	Colony 3	Colony 4	Colony 5
1st	2 hrs.	80° F.	−	−	−	−	−
2nd	" "	82° F.	−	−	−	−	−
3rd	" "	80° F.	−	−	−	−	+
4th	" "	90° F.	−	+	−	−	−
5th	" "		−	−	−	−	−
6th	" "	76° F.	−	−	−	−	−
7th	" "	72° F.	−	−	−	+	+
8th	" "		−	+	−	+	+
9th	" "	74° F.	−	+	+	+	+
10th	" "		−	+	−	+	+
11th	" "	82° F.	−	+	+	+	+
12th	24 hrs.		−	+	+	+	+
13th	" "		−	+	D	+	+

+ = Plant became infected.
− = Plant remained healthy.
D = Plant died.

insects do not become infective immediately after feeding on diseased plants. The incubation period varied from not less than 4 nor more than 8 hours to not less than 16 nor more than 20 hours in the different colonies.

One experiment was conducted in a laboratory where the temperature was maintained uniformly at 70° to 71° F. Two hundred and fifty nymphs were fed for 3 hours on diseased plants and then transferred in colonies, consisting of 50 insects each, to healthy plants at intervals of 3 hours. In this experiment all of the plants exposed to the colonies during the 1st, 2nd, and 3rd periods remained healthy. One plant of the set exposed during the 4th period of the series became infected; 2 plants exposed during the 5th period became infected; and 4 exposed during the 6th period became infected. All 5 of the plants exposed during the 7th period became infected. In the series of plants on which control colonies fed during the 6th period, one infection occurred. It is believed that this was due to accidental exposure to one or more infective aphids. The experiment again demonstrated an incubation period in the insects. At a uniform temperature of 70° to 71° F., the period was not less than 9 nor more than 24 hours.

One test was carried out in a dark cool cellar. The temperature of the cellar varied from 69° to 69.5° F. throughout the experiment. Five colonies, consisting of 100 nymphs each, were fed for 3 hours on diseased

Vicia faba plants and then transferred to a succession of healthy plants. The transfers to fresh plants were made at intervals of 3 hours. The infections resulting from exposure of healthy plants to the colonies in this experiment are shown in table 4. No infection occurred in any of the

TABLE 4.—*Infections obtained with 5 colonies of pea aphids in the nymphal stage when fed for 3 hours on diseased Vicia faba plants and then transferred to a succession of healthy plants under uniform conditions*

Period	Length of exposure	Approximate temperature during exposure	Succession of plants				
			Colony 1	Colony 2	Colony 3	Colony 4	Colony 5
1st	3 hrs.	69–69.5° F.	–	–	–	–	–
2nd	" "	" "	–	–	–	–	–
3rd	" "	" "	–	–	–	–	–
4th	" "	" "	–	–	+	–	–
5th	" "	" "	–	–	+	+	–
6th	" "	" "	–	–	+	+	–
7th	" "	" "	–	+	+	+	–
8th	24 hrs.	" "	+	+	+	+	–
9th	" "	" "	+	+	+	+	–

+ = Plant became infected.
– = Plant remained healthy.

plants exposed to the colonies during the 1st, 2nd, and 3rd periods. Colony 3 infected the plant on which it was placed during the 4th and each succeeding period of the series. Colony 4 infected the plant on which it was placed during the 5th and each succeeding period. Colony 2 infected the plant on which it was placed during the 7th and each succeeding period. Colony 1 became infective during the 8th period of the series. Colony 5 failed to infect any of the plants on which it was placed. This experiment again demonstrated an incubation period of the virus in the insects. The length of the incubation period as shown by infections varied even with colonies kept at a uniform temperature in the dark. At a temperature of 69° to 69.5° F., the incubation period varied from not less than 9 nor more than 15 hours to not less than 21 nor more than 48 hours in different colonies.

In the 6 experiments described above, approximately 2750 aphids in 30 colonies were fed for short periods on diseased plants and then transferred to several series of healthy plants. Of the 30 colonies of aphids, 4 failed to transmit the virus and one infected only a single plant. Two of the colonies infected the first plants on which they were placed after removal from diseased plants. These 2 colonies had fed for 6 hours on diseased plants in a greenhouse held at temperatures above 72° F. An incubation

period was definitely demonstrated for the remaining 23 colonies. In 20 of the colonies this period varied from not less than 9 nor more than 15 hours to not less than 21 nor more than 48 hours; in one colony a period of not less than 72 nor more than 96 hours was required; while in 2 colonies, held at temperatures of from 80° to 90° F., there was an incubation period of not less than 4 nor more than 10 hours.

RETENTION OF THE VIRUS BY THE PEA APHID

Two methods were used for the purpose of determining whether or not the virus is retained by the pea aphid for a considerable period of time. By the first method, pea aphids were fed on diseased plants and then transferred at frequent intervals to a succession of healthy susceptible plants. By the second method, pea aphids were fed on diseased plants and then transferred for a period of time to immune plants, after which they were transferred back to healthy susceptible plants.

Retention of the Virus by the Pea Aphid when fed on Susceptible Plants. *Vicia faba* was used in these experiments, since it had proved to be such a favorable plant on which to handle pea aphids. An objection to be considered, however, was the possibility that the insects might reacquire the virus from susceptible plants. Tests were, therefore, conducted in which it was found that freshly inoculated plants of *V. faba* did not serve as a source of virus for noninfective aphids for a period of at least 48 hours. From this it was concluded that by making transfers to fresh plants at daily or shorter intervals, the likelihood that the insects would reacquire the virus was remote.

In one experiment, 5 colonies of aphids in the nymphal stage were fed for 6 hours on diseased *Vicia faba* plants and then transferred to a succession of healthy plants. The nymphs were 1 to 3 days old when the experiment was started. After removal from diseased plants, 9 transfers to fresh plants were made at intervals of 2 hours, after which the insects were transferred to fresh plants every day. The colonies consisted of approximately 75 individuals each at the 10th transfer. During the 3rd week, the numbers began to dwindle and infections obtained in the 4th week were from a few surviving individuals. The results given by this test are shown in table 5. For some unknown reason, one of the colonies infected only 3 plants. This colony differed from the other colonies only in regard to the diseased plant upon which it had fed. Four of the colonies infected fresh plants each day for 17 days, and one of the 4 infected each of 9 plants on which it fed during the first day and continued to infect plants every day for 29 days. The experiment was discontinued after the 31st day, as only a few weakened individuals remained alive. These results demonstrated that pea aphids, when fed on diseased plants as small nymphs, acquired and retained the virus for periods up to 29 days.

TABLE 5.—*Inoculations with 5 colonies of pea aphids fed, when small nymphs, for 6 hours on diseased Vicia faba and then transferred to a succession of healthy plants, during the life of the insects*

Period	Length of exposure	Successions of plants				
		Colony 1	Colony 2	Colony 3	Colony 4	Colony 5
9 periods	2 hrs. each	8+	3+	5+	9+	1+
2nd day	24 hrs.	+	+	+	+	−
3rd day	" "	+	+	+	+	−
4th day	" "	+	+	+	+	−
5th day	" "	+	+	+	+	−
6th day	" "	+	+	+	+	−
7th day	" "	+	+	+	+	−
8th day	" "	+	+	+	+	−
9th day	" "	+	+	+	+	−
10th day	" "	+	+	+	+	−
11th day	" "	+	+	+	+	−
12th day	" "	+	+	+	+	−
13th day	" "	+	+	+	+	−
14th day	" "	+	+	+	+	−
15th day	" "	+	+	+	+	−
16th day	" "	+	+	+	+	−
17th day	" "	+	+	+	+	−
18th day	" "	+	+	+	+	−
19th day	" "	+	−	−	+	+
20th day	" "	+	+	−	+	−
21st day	" "	−	+	−	+	−
22nd day	" "	+	+	−	+	−
23rd day	" "	−	−	−	+	−
24th day	" "	−	+	−	+	−
25th day	" "	−	−	−	+	−
26th day	" "	−	−	−	+	−
27th day	" "	−	−	−	+	+
28th day	" "	−	−		+	−
29th day	" "	−	−		+	−
30th day	" "				−	−
31st day	" "				−	−

+ = Plant became infected.
− = Plant remained healthy.

To determine the effectiveness of single insects in retaining the virus, a record was made of the infections obtained from 25 individual pea aphids when fed for 1 or 2 days on diseased *Vicia faba* plants and then transferred every day to healthy plants. The record is shown in table 6.

Considerable variation was observed in the ability of different aphids to infect plants. Some of the aphids infected plants consistently on consecutive days. Others failed on alternate days, or for several days and

TABLE 6.—*Infections obtained with individuals of the pea aphid when fed on diseased Vicia faba plants and then transferred to a succession of healthy plants*

Period	Nymphs on diseased plant 24 hrs. 5/10–11/33					Alate adults on diseased plant 24 hrs. 7/8–9/33					Apterous adults on diseased plant 24 hrs. 7/8–9/33					Nymphs on diseased plant 48 hrs. 8/8–10/33					Nymphs on diseased plant 48 hrs. 8/8–10/33				
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
1st day	+	−	−	+	+	−	+	+	−	−	+	+	−	−	−	+	−	−	−	−	+	+	+	+	−
2nd day	+	+	+	+	−	+	+	+	+	+	+	+	+	−	−	+	−	−	−	−	+	+	−	+	−
3rd day	+	+	+	+	+	+	+	+	+	*	+	+	+	−	−	+	−	−	−	−	+	+	+	−	−
4th day	+	+	+	+	−	−	+	+	+	+	+	+	−	−	−	+	−	−	−	−	+	+	+	+	−
5th day	+	+	−	+	+	+	+	+	+	+	+	+	−	−	−	+	−	−	−	−	+	−	+	+	−
6th day	−	−	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	+	+	+	+	−
7th day	−	+	+	+	−	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	+	−	+	−	L
8th day	D	+	+	+	−	+	+	+	+	+	+	+	+	−	−	+	−	−	−	−	+	+	−	+	
9th day		+	+	−	+	+	+	+	+	+	+	+	+	−	−	+	−	−	−	−	−	−	+	+	
10th day		+	−	−	−	+	+	+	−	−	−	−	−	−	−	+	−	−	−	−	+	L	−	−	
11th day		−	−	+	+	−	−	+	+	−	−	−	−	−	−	+	−	−	−	−	+		+	+	
12th day		+	+	−	+	−	−	+	+	+	−	+	−	−	−	−	−	−	−	−	+		−	−	
13th day		−	−	−	−	−	+	+	+	+	A	A	A	A	A	+	−	−	−	−	+		−	−	
14th day		+	−	−	−	−	+	−	−	−						+	−	−	−	−	+		L	+	
15th day		−	D	−	−	−	+	+	+	+						+	−	−	−	−	+			+	
16th day		+		−	−	A	A	A	A	A						−	−	−	−	−	−			−	
17th day		−		−	−											A	A	A	A	A	A			L	
18th day		−		D	−																				
19th day		−			−																				
20th day		+			−																				
21st day		D			−																				
22nd day					−																				
23rd day					+																				
24th day					−																				
25th day					−																				
26th day					D																				

+ = Plant became infected.
− = Plant remained healthy.
* = Plant dead from fumigation.
D = Insect dead.
L = Insect lost.
A = Discontinued with insects still alive.

then again infected plants. The longest observed time in which a single aphid remained infective was 23 days. This result was obtained with an insect that was fed on a diseased plant when 3 days old and that infected a plant on which it was placed 23 days later.

The most consistent performance was observed in 5 individuals that were winged adults at the time they were placed on diseased plants. In a period of 15 days, these 5 aphids infected 57 of the 75 plants on which they were placed. Four of the 5 infected plants on the 15th day. One infected 14 of the 15 plants on which it was placed, failing only on the 14th day. These insects had completed reproductive activity and appeared sluggish and weakened during the last few days. They were, therefore, not carried beyond the 15th day.

It was found that some of the insects, after being infective for a time, failed to infect plants in later periods, while others were infective until within a day or two of death. The tests did not show whether this failure was due to a loss of the virus by the insects or to some factor, such as a weakened condition, that might affect the feeding habits of the insects. These tests confirmed the results obtained in experiments with colonies. They also demonstrated the effectiveness of single insects as carriers of the disease. The retention of the virus by single insects was shown for periods up to 23 days.

Retention of the Virus by the Pea Aphid when fed on Immune Plants. It was believed that the possibility of aphids reacquiring the virus from plants on which they were allowed to feed would be reduced to a minimum if immune plants were used. Information, however, was meager concerning plants that were at the same time immune from the virus and satisfactory as food for the aphids. Among the plants that had failed to become infected after inoculation with this virus by the pea aphid was the Spanish peanut. It appeared also to be satisfactory as a food plant for this aphid. The Spanish peanut was, therefore, used in one experiment on retention of the virus by the pea aphid. Two hundred and fifty nymphs were placed on diseased *Vicia faba* plants and allowed to feed for 2 days. They were then placed for one day on 10 healthy *V. faba* plants before transfer to peanuts. They remained on the peanut plants for 7 days, being disturbed only once in order to remove accumulating progeny. When removed from the peanuts, they were transferred successively to 2 lots of 10 plants each of *V. faba*. No evidence of infection became apparent in any of the peanut plants, though they were kept under observation in a greenhouse for 2 months. All of the *V. faba* plants in the 2 lots became infected, demonstrating that the pea aphids had retained the virus for 7 days when fed on an immune plant. Experiments have not as yet been carried out that would show retention of the virus by the pea aphid when fed for more extended periods on immune plants.

TRANSMISSION BY OTHER INSECTS THAN THE PEA APHID

In order to determine whether other insects than the pea aphid can transmit pea mosaic, tests were made with the bean aphid, *Aphia rumicis* L., and the potato aphid, *Macrosiphum gei* (Koch).

The bean aphid was observed in abundance at times on *Vicia faba* in the field, and the possibility was suggested that it might carry the disease. Colonies of the bean aphid were maintained continuously on *V. faba* plants in a greenhouse. For the purpose of determining whether or not they would transmit the disease, bean aphids that had developed on diseased *V. faba* were transferred to small healthy plants. From 25 to several hundred insects were transferred to each plant and allowed to feed for at least 2 days. One lot of 20 plants was placed in a screened cage for one week beside diseased plants on which aphids were feeding, to permit of natural migration from diseased to healthy plants. In all, 100 plants of *V. faba* were exposed to bean aphids that had developed on diseased plants. Since no infection appeared in any of these plants, it is concluded that the bean aphid is not able to transmit the disease.

Tests with the potato aphid as a possible vector of the mosaic were undertaken, because it is closely related to the pea aphid and is known to feed at times on peas and clovers. In these tests the pink asexual form of the potato aphid was used rather than the green form, in order that it might be readily distinguished from the pea aphid. Colonies were maintained on tomato plants in screened cages.

In one test potato aphids were fed for 2 days on diseased *Vicia faba* plants and then transferred to 20 healthy plants. They remained on the healthy plants one day and were then transferred to a second lot of 20 healthy plants and allowed to feed for one day. Twenty to 30 aphids were placed on each healthy plant. Of the 20 plants exposed the first day, 19 became infected, and of the 20 plants exposed the 2nd day, 19 again became infected. The experiment demonstrated that the potato aphid, as well as the pea aphid, is capable of transmitting the disease. No attempt has been made to determine whether or not the pea-mosaic virus requires an incubation period in the potato aphid before it is transmitted by this vector.

Experiments to determine whether or not other insects are capable of transmitting pea mosaic have not been undertaken. The studies reported above, however, definitely establish the fact that the disease is transmitted by both the pea aphid and the potato aphid, and that it is not transmitted by the bean aphid. It is concluded, therefore, that, although the disease can be transmitted by more than one species of insect, the ability to transmit is nevertheless restricted to certain species.

TRANSMISSION BY MECHANICAL METHODS

To determine if pea mosaic can be transmitted by mechanical methods, as well as by the pea aphid and potato aphid, a total of 772 *Vicia faba* plants and 150 garden pea plants were inoculated with juice expressed from diseased *V. faba* plants. Eighty garden pea and 20 *V. faba* plants were inoculated with juice expressed from diseased garden peas. These were grown beside control plants that remained healthy. Only 3 of the inoculated plants became infected. Of the 3 that became infected, one was in a lot of 40 plants inoculated by punctures with 000 insect pins moistened with juice from diseased *V. faba* plants. Since infection did not appear until 21 days after inoculation, it was felt that contamination might have occurred. Two of the plants that became infected were in a lot of 100 *V. faba* plants inoculated with expressed juice from diseased *V. faba* plants to which norit was added at the rate of 1 part in 20. The juice was rubbed into the surface of the leaves with bandage gauze. Infection appeared 13 days after inoculation. The number of infections obtained from these inoculations was so small as to be unsatisfactory for drawing a final conclusion as to transmissibility by mechanical means. They did demonstrate that the transmission of this mosaic by the usual mechanical methods is extremely difficult.

DISCUSSION

The experiments presented in this paper have shown that the pea aphid is not able to infect healthy plants immediately after first feeding on diseased plants, but that an interval, referred to as an incubation period of the virus in the insect, must elapse. However, once the insect acquires the virus, it is retained for considerable periods of time. These results indicate that the transmission of this disease by the pea aphid is similar as regards incubation period and retention to the transmission of curly top of beet, aster yellows, and streak of maize by leaf hoppers.

The nature of the incubation period and means by which the virus is retained in the insect are not known. It is possible that the virus may multiply or pass through a stage of development in the insects. On the other hand, the incubation period may merely represent the time required for the virus to diffuse through the body fluids or tissues to reach a point where it may be injected into the plant by the feeding of the insects.

Whatever the explanation, it is of interest that this mode of transmission appears to differ from that shown to occur in the transmission of cucumber mosaic and yellow dwarf of onions by aphids, in which transmission is without an interval or incubation period and in which retention of the ability to infect by the insects is for only a brief period.

Curly top of beet, aster yellows, and streak of maize are known to be

carried only through the agency of single species of leaf hopper. It is of interest that in the case of pea mosaic, in which a similar mode of transmission has been found, 2 species of aphids, the pea aphid and the potato aphid, have been shown to be carriers. The ability to transmit the disease is nevertheless restricted to certain species. The bean aphid is not able to spread the disease. It is also of interest that pea mosaic is difficult to transmit by mechanical methods.

SUMMARY

This paper presents the results of experiments that demonstrate an incubation period of pea mosaic in the aphid, *Macrosiphum pisi* (Kaltenbach).

By exposing successions of healthy *Vicia faba* plants to colonies of pea aphids that were allowed to feed for short periods on diseased plants, an incubation period of the virus in the aphids was shown to exist. This was definitely demonstrated for 23 colonies. In 20 of the colonies the period varied from not less than 9 nor more than 15 hours to not less than 21 nor more than 48 hours; in one colony a period of not less than 72 nor more than 96 hours was required; while in 2 colonies, held at temperatures of from 80° to 90° F., there was an incubation period of not less than 4 nor more than 10 hours.

Pea aphids that acquire the virus after feeding on diseased plants retain it for considerable periods of time. Retention of the virus was demonstrated with single insects, as well as with colonies. One colony retained the virus over a period of 29 days.

The potato aphid, as well as the pea aphid, transmits the disease, but the bean aphid does not.

It was found difficult to transmit this mosaic by mechanical methods.

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EFFECT OF NITROGEN SUPPLY ON HOST SUSCEPTIBILITY TO VIRUS INFECTION[1]

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It has long been known from studies dealing with host susceptibility to parasitic infection that the environmental factors, mineral nutrition, light, temperature, and humidity, influence the host response to infection. In the field of plant-virus investigations several of these factors have been studied, but a review of the literature shows that very little consideration has been given to the possible effects of mineral nutrition on host susceptibility to virus infection. The object of this paper is to present the results of experiments dealing with the influence of variations in the nitrogen supply on the susceptibility of 3 different hosts to tobacco mosaic.

REVIEW OF LITERATURE

The influence of plant succulence in altering host tolerance for virus diseases has been recognized since 1922. Observations by Dickson (4) on tobacco mosaic, Elmer (6) on tomato mosaic, Kraybill and Eckerson (12) on tomato mosaic, Kirkpatrick (11) on leaf curl of cotton, Drake and coworkers (5) on yellow dwarf of onion, and Carter (3) on yellow spot of pineapple indicate that there is a definite relationship between host succulence and susceptibility to infection. Samuel and Bald (17) found that dormant seedlings of *Nicotiana glutinosa* L. were considerably more resistant to infection with tobacco-mosaic virus than vegetative seedlings.

Little work has been recorded on the factors underlying host succulence. The few brief reports so far published on nitrogen nutrition and host susceptibility to virus infection may be summarized briefly as follows: Schultz and Folsom (18) observed that during the first part of the season the percentage of mosaic on potatoes was lowered to some extent by high nitrogen and high potassium fertilizers. On the other hand, when healthy potato plants were interspersed with mosaicked plants, Janssen (9) found that the lowest incidence of mosaic was almost uniformly among the plants receiving little or no nitrogen. In a similar test with leaf roll of potato, he found that the resistance of plants in low-nitrogen plots was even more conspicuous. Quanjer (14) concluded from his investigations and those of his collaborators that pseudo-net-necrosis spreads more readily to healthly plants when the nitrogen supply is high. Ainsworth (1) observed on permanent soil plots receiving the same fertilizer treatment each year that the

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number of tomato plants affected by tomato stripe on a plot receiving complete fertilizer was 5.0 per cent, but when nitrogen was omitted from the complete fertilizer it was only 1.6 per cent. Samuel and Bald (17) found that occasional watering with ammonium phosphate considerably increased the susceptibility of *Nicotiana glutinosa* to tobacco-mosaic virus.

The only detailed experiment published to date on the effect of nutrition on host susceptibility to virus diseases is that of Volk (20) who grew tomato and tobacco plants in nutrient sand cultures. The tomato plants were inoculated with spot-mosaic virus (Fleckmosaik) when they were 8 weeks old. The tobacco was inoculated at the pre-flower stage by injecting expressed sap from a mosaic-diseased tobacco plant under the epidermis of the lowermost leaf. His results, with respect to nitrogen nutrition, show that the percentage of diseased plants is increased with increasing amounts of nitrogen. The effect of nitrogen on growth was not considered.

MATERIALS AND METHODS

The experiments reported in this paper were planned to study the effect of nitrogen supply on growth and host susceptibility to tobacco mosaic. The host plants were *Nicotiana glutinosa* L., *N. tabacum* L. var. Turkish, and *Phaseolus vulgaris* L. var. Early Golden Cluster. Growth was measured by recording the green weight and height of representative plants in each test at time of inoculation. Since the resultant graphs of these two growth criteria proved to be similar, only the green weight data are here presented. Susceptibility of plants receiving different amounts of nitrogen was determined by counting the number of lesions per 100 pin punctures on tobacco and *N. glutinosa* and by the number of lesions per leaf on bean plants.

Ordinary green tobacco-mosaic virus, designated by Johnson (10) as tobacco virus 1, was used to infect *Nicotiana glutinosa* and bean as it forms necrotic primary lesions on both of these hosts. The bean variety used, Early Golden Cluster, was shown by Price (13) to produce large numbers of primary lesions when inoculated with green tobacco-mosaic virus. Since much is already known concerning the reaction of Turkish tobacco to tobacco mosaic, this variety was chosen as the third host, even though the chlorotic primary lesions of tobacco mosaic on tobacco are often obscure and have to be stained with potassium iodide-iodine solution (8) to make them sufficiently distinct to be counted. On tobacco a yellow tobacco-mosaic virus, Johnson's tobacco virus 6, was used in preference to the ordinary green tobacco-mosaic virus previously mentioned because of its more drastic action on chloroplasts. Ordinary green tobacco mosaic (8) exerts only a slight inhibitory action on starch formation, whereas yellow tobacco mosaic (16) inhibits starch formation completely. For this reason, lesions of yellow tobacco mosaic are easier to demonstrate by the iodine method. In

order to record the number of lesions on tobacco, the inoculated leaves were removed from the plants late in the afternoon and placed in 95 per cent ethyl alcohol to kill the cells and to dissolve out the chlorophyll. The leaves were then stained in a weak aqueous solution of iodine in potassium iodide. Primary lesions appeared as small yellow spots against a dark background of healthy tissue. The two strains of tobacco-mosaic virus, green and yellow, were obtained several years ago from James Johnson and since that time have been passed through tobacco plants many times before being used in the experiments here reported.

Bean seedlings were inoculated by lightly rubbing the entire upper surface of the leaves with a small cheesecloth pad saturated with a 1–100 dilution of freshly extracted tobacco-mosaic virus. The juice was prepared by grinding several leaves of a diseased tobacco plant in a sterile mortar and then diluting with water. Tobacco and *Nicotiana glutinosa* were inoculated by the pin-puncture method, as described by Holmes (7). A set of 20 small black enamel insect pins, size No. 000, were bound between pot labels that served as a handle. With such a set, it was possible to make 20 pricks by each thrust. If the punctures were made interveinally, very little pressure was needed to force the pins through the leaf. The pins were dipped in freshly extracted undiluted juice from a diseased plant and then pricked into the test plant.

Beans were planted, 3 in each 4-inch pot, in washed, white quartz sand. Seeds of tobacco and *Nicotiana glutinosa* were germinated in sand and the seedlings kept moist with a complete nutrient solution. When the seedlings had 3 or 4 small leaves and were about 2 cm. in height, they were potted in 4-inch pots in a soil that was very low in fertility. Although sand would have been preferable to soil for the Nicotiana species, the latter was used because of the time required to feed sand cultures daily for 5 weeks. Since beans were usually on nutrient treatment for only 8 days, they could be cultured in sand without involving an undue amount of labor.

The nutrient culture technique was similar with all three hosts. The plants were fed 100 cc. per pot with a modified Shive's (19) 3-salt solution as a basic nutrient. To this solution was added the calculated amount of nitrogen as $Ca(NO_3)_2$ for each treatment. Ammonium nitrate was not used as a source of nitrogen because both tobacco and bean are somewhat sensitive to ammonium nitrogen. Ammonium nitrogen also masks to some extent the primary lesions on tobacco, as shown by the iodine staining technique. The composition of the basic solution, which was well balanced with regard to all the essential elements except nitrogen, is given in table 1. The tobacco and *Nicotiana glutinosa* plants in soil were fed twice each week; the beans in sand were fed daily.

Two or more tests extending over several seasons of the year were carried out with each host. During the experimental period the tempera-

TABLE 1.—*Composition of basic minus-nitrogen nutrient solution*

Salts used	Molecular proportions	Partial volume-molecular concentrations[a]
KH_2PO_4	3	.0036
$CaCl_2$	2	.0024
$MgSO_4$	3	.0036

[a] Total osmotic concentration value approximately 0.5 atmosphere.

ture of the greenhouses was held between 70° and 75° F. at night and between 70° and 80° F. during the day. Although no aphids or other insects were noticed, the greenhouses were fumigated weekly with nicotine to avoid any possible contamination by insect transmission. In all tests the pots were set in saucers and spaced to avoid crowding of foliage. For clearness, the results obtained with each host will be presented separately.

INFLUENCE OF NITROGEN ON SUSCEPTIBILITY OF NICOTIANA GLUTINOSA TO GREEN TOBACCO MOSAIC

The plants were inoculated when the blossom buds first began to show. At this stage of growth the number of leaves per plant suitable for inoculation ranged from 4 in the slow-growing plants to 10 in the most vegetative ones. One hundred pin punctures were made interveinally in each leaf. The final record of the number of necrotic primary lesions visible was made on the 5th day after inoculation.

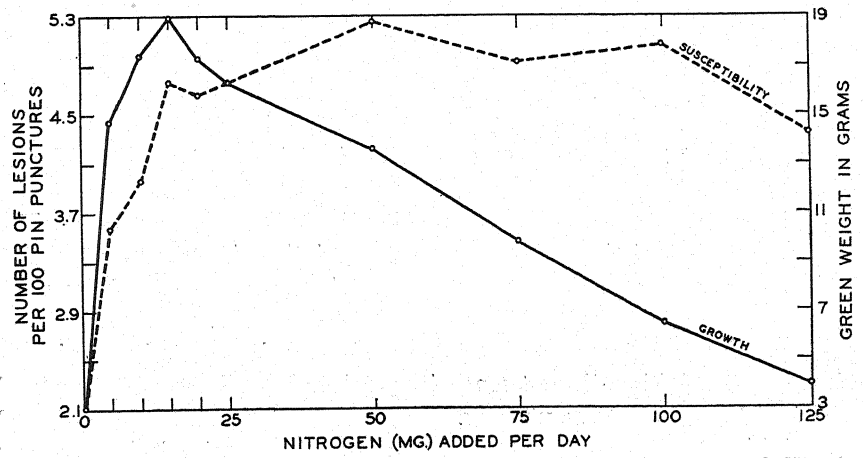


FIG. 1. Influence of nitrogen supply on growth and susceptibility of *Nicotiana glutinosa* to green tobacco mosaic. The solid line shows the average green weight of plants at time of inoculation; the broken line, the number of lesions per 100 pin punctures.

The average results of two tests with *Nicotiana glutinosa* are shown graphically in figure 1. The solid line represents the average green weight at time of inoculation of the plants receiving the amounts of nitrogen indicated along the base line. The broken line represents the susceptibility of similar plants to green tobacco mosaic. From these graphs it is evident that growth is influenced very markedly by nitrogen. Maximum growth occurs at a relatively low nitrogen level (15 mg. per day) and rapidly falls off as nitrogen is increased. High susceptibility, on the other hand, occurs over a wide range of nitrogen levels (15–100 mg. nitrogen per day). Thus, susceptibility is not definitely correlated with rapidity of growth.

INFLUENCE OF NITROGEN ON SUSCEPTIBILITY OF BEAN TO GREEN TOBACCO MOSAIC

As soon as the plumules became visible between the cotyledons, usually 6 or 7 days after planting, the nutrient treatments were started. About 6 days later, when the first true leaves were fully developed, the plants were trimmed by removing the growing tip of each to prevent intertwining during subsequent growth, and then inoculated. Small necrotic lesions appeared on the leaves the 2nd or 3rd day after inoculation. The lesions were usually counted on the 4th or 5th day after inoculation when even the smallest were large enough to be seen easily.

On plants receiving low amounts of nitrogen, the lesions appeared as reddish brown spots 1 to 2 mm. in diameter, with grayish brown necrotic centers. With high nitrogen nutrition the lesions were considerably smaller, ranged in color from dark reddish brown to almost black, and had no light-colored centers. The lesions appeared earlier at low and medium nitrogen levels than at high levels.

The bean plant makes such rapid growth that marked response to nutritive treatment, both in growth and susceptibility, is observed after a few days of nutrient treatment. The results obtained in 4 tests on the effect of nitrogen nutrition on the susceptibility of bean to tobacco mosaic are presented in table 2. It is readily seen from this table that the susceptibility of the bean is definitely influenced by variation in nitrogen supply. Maximum growth, as measured by green weight, was produced by moderately low amounts of nitrogen, between 20 and 60 mg. per day. Figure 2 shows typical plants at several different nitrogen levels at time of inoculation. Susceptibility did not reach its maximum until the nitrogen level was raised to 80 or 100 mg. per day. This relationship between growth and susceptibility as shown by the bean at various nitrogen levels is expressed graphically in figure 3. Data for these graphs were taken from table 2. The solid line represents the average green weight, in grams, of representative plants at time of inoculation. The broken line represents the average

TABLE 2.—*Response shown by bean to various nitrogen treatments and the effect of such treatments on the host susceptibility to green tobacco mosaic*

Nitrogen added per day (mg.)	Green weight (gm.) at time of inoculation				Number of lesions per leaf				
	Test No. 2	Test No. 3	Test No. 4	Average	Test No. 1	Test No. 2	Test No. 3	Test No. 4	Average
0	2.4[a]	2.3[a]	2.1[a]	2.27	1[b]	1[b]	9[b]	15[b]	7
10					2	7	9	41	15
20	2.9	2.6	2.7	2.73	7	14	8	39	17
40	2.9	2.5	2.5	2.63	11	25	11	72	30
60	3.0	2.5	2.5	2.67	17	50	12	99	45
80	2.6	2.3	2.5	2.47	26	53	13	123	54
100	2.4	2.4	2.2	2.33	28	42	29	101	50
125					39	30	17	82	42
150	2.2	2.1	2.1	2.13	28	20	12	60	30
175					30	23	18	72	36
200	2.2	2.1	1.8	2.03	13	19	15	41	22
225						15	13	33	20
250		2.1	1.8	1.95		13	6	24	14
300	1.7	1.7	1.5	1.63		11	1	18	10

[a] Each value represents the average green weight of 32–43 plants.
[b] Each value represents the average number of lesions per leaf of 81–119 plants.

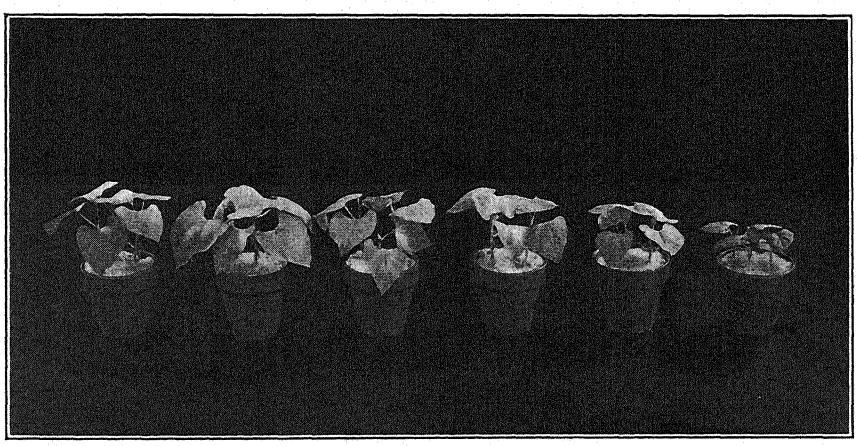


FIG. 2. Typical bean plants that have received 0, 20, 80, 100, 200, and 300 mg. of nitrogen per day, respectively, from left to right, photographed at time of inoculation. Note that after the 2nd pot from the left, the plants become smaller as the nitrogen level is raised. The plants in the 2 middle pots proved to be most susceptible.

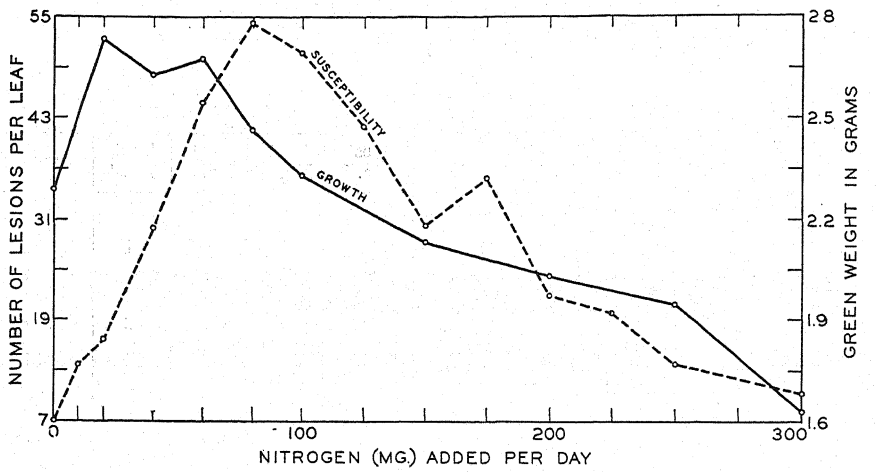


FIG. 3. Effect of nitrogen on growth and susceptibility of bean to green tobacco mosaic. The solid line shows the average green weight of plants at time of inoculation; the broken line, the average number of lesions per leaf when nitrogen is added as indicated along the base line.

number of primary lesions per leaf when the plants were supplied with the amounts of nitrogen indicated along the base line.

INFLUENCE OF NITROGEN ON SUSCEPTIBILITY OF TOBACCO TO YELLOW TOBACCO MOSAIC

After approximately 4 weeks of nutrient treatment when the tobaccos had reached the 12- or 13-leaf stage of development, representative plants from each treatment were inoculated with yellow tobacco-mosaic virus by pin punctures. The number of punctures per leaf ranged from 60 in the youngest leaf (about 4 cm. long) to 280 or 320 in the oldest leaf, depending on its size. On the 5th day after inoculation, before the primary lesions had fused or systemic symptoms had appeared, the youngest leaves were removed and treated by the iodine method as modified by Samuel (16) for yellow tobacco mosaic. Since the expression of primary symptoms is delayed in old tissue, the remaining leaves were not removed until the 7th day after inoculation.

Results of 3 tests on the effect of nitrogen on growth are recorded in table 3. From these data it is readily apparent that maximum growth, as measured by green weight, was produced when very small amounts of nitrogen, between 10 and 15 mg. per plant per day, were added. Figure 4 shows typical tobacco plants that have received 0, 10, 20, 75, and 125 mg. of nitrogen per day, respectively, photographed at time of inoculation. With large applications of nitrogen, growth was greatly retarded. When more than

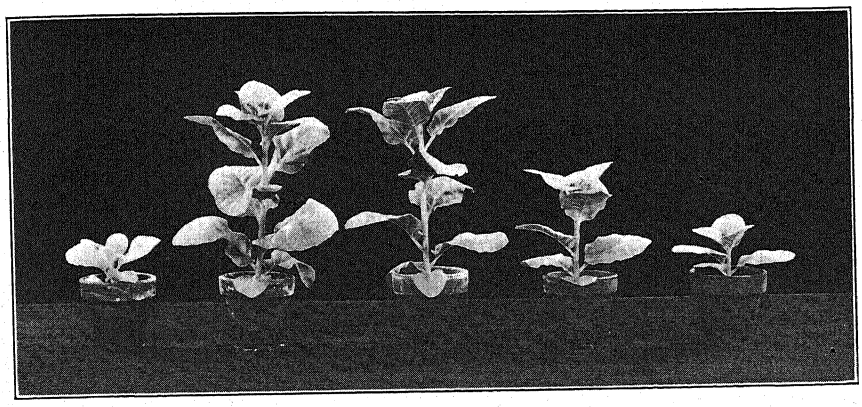


FIG. 4. Typical tobacco plants that have received 0, 10, 20, 75, and 125 mg. of nitrogen per day, respectively, from left to right, photographed at time of inoculation. The 2 plants on the right were the most susceptible.

125 mg. of nitrogen per day were added, most of the plants died. Plants receiving large quantities of nitrogen were much darker in color than those that made the fastest growth. Plants that received no nitrogen made little growth. The leaves were stiff, leathery in texture, and yellowish in color. Several of the lowermost leaves died and dried up. In no other treatment did the plants show any evidence of nitrogen starvation.

TABLE 3.—*Effect of nitrogen on growth and susceptibility of tobacco to yellow tobacco mosaic*

Nitrogen added per day (mg.)	Green weight (gm.) at time of inoculation				Number of lesions per 100 pin punctures			
	Test No. 1	Test No. 2	Test No. 3	Average	Test No. 1	Test No. 2	Test No. 3	Average
0	11.0[a]	5.6[a]	2.0[a]	6.2	2.0[a]	1.9[a]	2.5[a]	2.1
5	27.0	23.0	27.5	25.8	4.6	2.4	4.5	3.8
10	38.4	31.0	31.2	33.5	4.7	2.9	4.7	4.1
15	38.4	30.8	33.0	34.1	4.3	3.6	4.0	4.0
20	34.2	27.6	29.4	30.4	4.6	5.0	4.3	4.6
25	33.9	26.0	28.8	29.6	5.5	4.2	4.5	4.7
50	23.9	17.4	18.2	19.8	5.5	5.0	5.4	5.3
75	13.4	11.2	10.4	11.7	5.7	5.8	7.3	6.3
100	6.6	5.2	5.8	5.9	3.5	7.7	7.4	6.2
125	2.6	3.8	3.7	3.4	3.5	4.4	7.6	5.2

[a] Average of 5 plants.

Table 3 also shows results obtained by inoculating sets of tobacco plants, similar to those harvested for green weight determinations, with yellow tobacco-mosaic virus. It is seen that susceptibility to virus infection gradually rises with increasing amounts of nitrogen up to a definite level (75–100 mg. nitrogen per day), beyond which susceptibility falls off gradually. No appreciable difference could be noted in the appearance of the primary lesions with any of the various nitrogen treatments.

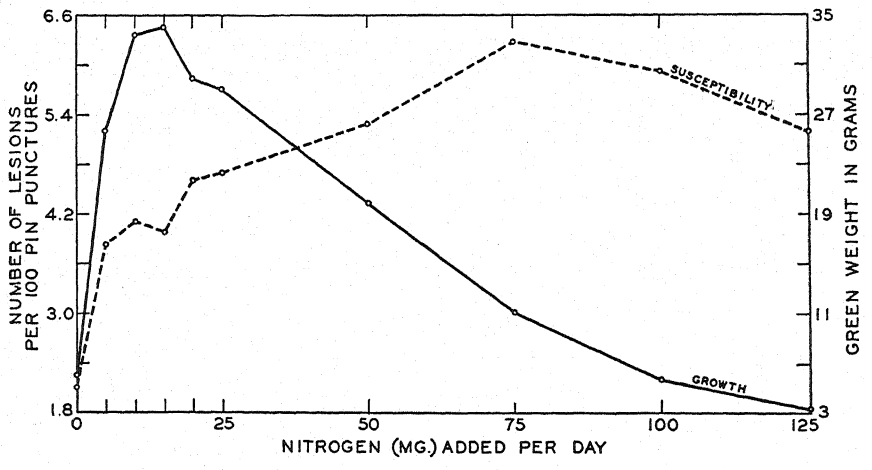


FIG. 5. Influence of nitrogen supply on growth and susceptibility of tobacco to infection with yellow tobacco mosaic. The solid line shows the average green weight of plants at time of inoculation; the broken line, the number of lesions per 100 pin punctures when nitrogen is added as indicated along the base line.

The relationship between growth and host susceptibility of tobacco as influenced by nitrogen nutrition is expressed graphically in figure 5. The solid line represents the average green weight at time of inoculation of plants supplied with nitrogen at the various levels. The broken line represents the susceptibility of similar plants to yellow tobacco mosaic. These graphs show that, with tobacco, highest susceptibility does not occur at nitrogen levels favoring the most rapid growth; in fact, the nitrogen levels producing maximum susceptibility exert a retarding influence on growth.

The question arises as to whether the response in growth and susceptibility was due to nitrogen or to calcium, since nitrogen was added as $Ca(NO_3)_2$ and calcium increased simultaneously with nitrogen. In order to answer this question, another test was carried out in which nitrogen only was varied by using NH_4NO_3 instead of $Ca(NO_3)_2$ as a source of nitrogen, and calcium was supplied as $CaCl_2$. The symptoms of ammonium toxicity were not severe enough to interfere materially with the lesion count at the end of the experiment. The results of this test are expressed graphically

in figure 6. The solid line shows the average green weight at time of inoculation; the broken line, the susceptibility of representative plants when in-

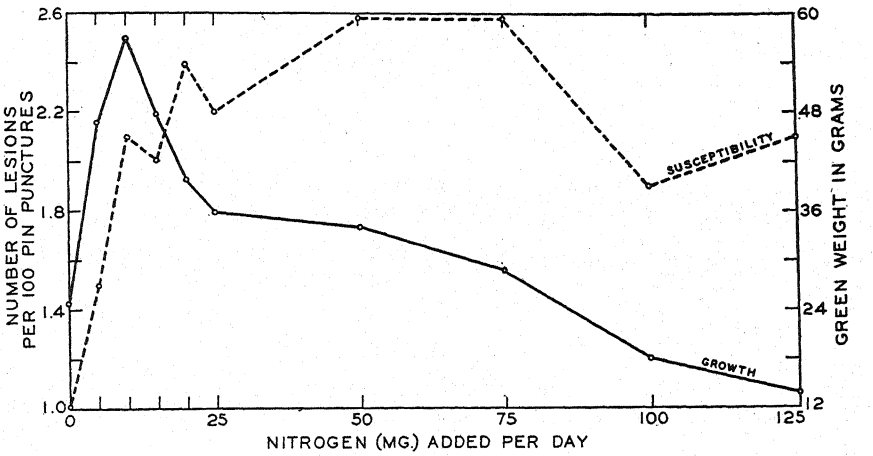


FIG. 6. Influence of nitrogen supply on growth and susceptibility of tobacco to yellow tobacco mosaic when the calcium supply is held constant and only nitrogen is varied. The solid line shows the average green weight at time of inoculation; the broken line, the number of lesions per 100 pin punctures. Five plants were used in obtaining data for each point on the graphs.

TABLE 4.—*The susceptibility of various portions of tobacco plants as influenced by nitrogen*

Nitrogen added per day (mg.)	Number of lesions per 100 pin punctures[a]			
	Upper portion	Middle portion	Lower portion	Entire plant
0	4.3	1.7	1.3	2.1
5	8.7	3.6	1.7	3.8
10	10.1	3.0	2.0	4.1
15	10.5	2.8	1.6	4.0
20	11.8	3.5	2.0	4.6
25	10.9	3.8	2.4	4.7
50	11.8	4.2	2.9	5.3
75	11.1	6.2	3.7	6.3
100	9.0	5.8	4.6	6.2
125	8.4	4.7	3.4	5.2

[a] Each value represents the average of 15 plants.

oculated with yellow tobacco-mosaic virus. Each point on the graphs represents the average value obtained with 5 plants. The graphs in figure 6 correspond very closely with those in figure 5. This similarity indicates, therefore, that the trend of the graphs in figure 5 is due primarily to nitrogen variation and not to variation in the amount of calcium.

It is of interest to note the extent to which the susceptibility of leaves of various ages on the same plant is affected by varying the nitrogen supply. In table 4 are recorded data showing the susceptibility of leaves of the upper, middle and lower portions of the plant at the various nitrogen levels. The results show that the susceptibility of all the leaves on the plant is altered by changing the nitrogen level. The maximum susceptibility in the upper portion is reached at a lower nitrogen level than is reached by the middle portion, and maximum susceptibility in the lowermost portion is reached only at a still higher level. It is also apparent that the upper leaves at any nitrogen level are markedly more susceptible than the middle leaves, and these in turn are more susceptible than the lowermost leaves. There is no change in this relationship between leaves at any nitrogen level tested.

DISCUSSION

The importance of salt nutrition in affecting the susceptibility of plants to bacterial and fungal diseases has been emphasized by many investigators. In a brief review of the literature on this subject, Raines (15) has pointed out that a general correlation exists between host vigor and susceptibility. In other words, a vegetative host is more susceptible to parasitic infection than a nonvegetative or dormant host. The experiments reported in this paper do not extend this general conclusion to the viruses tested.

Experiments on 3 different hosts, one of which is widely separated taxonomically from the family Solanaceae, show quite definitely that the correlation between host vigor, as measured by the rate of growth, and host susceptibility to virus infection may be changed by the nitrogen nutrition of the host. That is to say, with 15 mg. of nitrogen per day, an amount that produced the best growth of tobacco, the plants were only 2/3 as susceptible as they were when 5 times as much nitrogen was added. At this high nitrogen level, however, growth had fallen off nearly 66 per cent. Therefore, in order to get high susceptibility, more nitrogen than is required for good growth must be added. This same relation, although varying slightly in degree, depending on weather conditions and season, was found to hold true with both *Nicotiana glutinosa* and bean. Each experiment was repeated one or more times and not a single exception was noted in any of the tests.

Some other factor, apparently, is more effective than rapidity of growth

in determining the incidence of infection. In the light of present knowledge, it is impossible to advance any satisfactory explanation as to the nature of this secondary factor, but it undoubtedly is related in some way to the physiological processes going on within the living plant cell.

Comparatively little is known with certainty regarding the type of action that nitrogen exerts in regulating host susceptibility to virus infection. Since young tissue is more susceptible to virus infection than old tissue, it is conceivable that cell activity may be a factor for successful virus infection. A study of the nitrogen metabolism within the host may shed some light on the way in which nitrogen influences host susceptibility. It has been shown by Archbold (2) that cell activity as indicated by respiration is relatively rapid when the content of assimilated nitrogen is high. Thus, the increase in infectivity with increasing amounts of nitrogen may be due to an increase in cell activity. It is also conceivable that susceptibility to virus infection may be dependent upon a high concentration of some one of the organic nitrogenous fractions within the plant.

The most generally accepted view is that the virus particle can enter the plant only through an injured cell that is able to recover. Since this repair process calls for synthesis of new protoplasmic material, a deficiency of any substance or element required in this process would indirectly lower the susceptibility of the host. The presence of such a material may be more dependent on some physiological condition within the plant than on host vigor itself.

Further work is necessary to show why growth falls off with increasing amounts of nitrogen. Since cell activity is increased by high nitrogen, it is possible that the carbohydrates in the plant tissue may be used up in respiration before they can be utilized in protein formation. Furthermore, it is possible that protein synthesis may be stopped at some stage in the process by high nitrogen nutrition.

SUMMARY

A study was made of the effect of nitrogen supply on host susceptibility to virus infection.

Turkish tobacco plants grown in soil at different nitrogen levels were inoculated with yellow tobacco-mosaic virus (Johnson's tobacco virus 6) by the pin-puncture method. Susceptibility was measured by the number of lesions per 100 pin punctures. Plants of *Nicotiana glutinosa* L. grown under similar conditions were inoculated with ordinary green tobacco-mosaic virus (Johnson's tobacco virus 1).

In similar tests, Early Golden Cluster beans were grown at various nitrogen levels in sand cultures and inoculated by rubbing the leaves with green tobacco-mosaic virus. Susceptibility was measured by the average number of primary lesions per leaf.

Results from the study of these three hosts indicate quite conclusively, first, that there is a definite correlation between host nutrition and host susceptibility to virus infection, and secondly, that host susceptibility is not governed mainly by host vigor as judged by the rate of growth, but by some other limiting factor the nature of which is at present unknown.

Plants which made the most rapid growth were considerably less susceptible than those in which growth was retarded by excess nitrogen. Thus, in order to get highest susceptibility more nitrogen than is required for good growth must be added. Small additions of nitrogen increase plant growth more than plant susceptibility.

In tobacco the susceptibility of all the leaves on the plant is altered by changing the nitrogen level. The upper leaves at any nitrogen level are markedly more susceptible than the middle leaves, and these in turn are more susceptible than the lowermost leaves. There is no change in this relationship between leaves at any nitrogen level tested.

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ANTISEPTIC SOLUTIONS AND ANTISEPTIC ADHESIVE TAPE IN RELATION TO CONTROL OF HAIRY ROOT, CROWN GALL, AND OTHER OVERGROWTHS ON NURSERY APPLE TREES[1]

A. J. Riker, S. S. Ivanoff, and F. B. Kilmer

(Accepted for publication May 31, 1934)

INTRODUCTION

The control of the various types of overgrowth that occur at the union of piece-root apple grafts has challenged the attention of plant pathologists for a number of years. Certain distinct progress in this direction has been made by various writers whose work is reviewed by Riker *et al.* (8). In this paper attention has been directed to the advantages of an adhesive-tape wrapper as one of the most promising means of reducing the percentage of both parasitic and nonparasitic graft knots at the union. As already pointed out (7), (8), certain failures of this tape to provide fully adequate control were found to be either because of the incidence of infection at the time of grafting, or because of the initiation of infection after the wrapper had disintegrated during the latter part of the first year, or during subsequent years in the nursery. Obviously the situation could be influenced little except by reducing the source of inoculum through manipulations during grafting time. The prevention of infection at the time of grafting, however, provided an opportunity for the application of control measures.

Infection at time of grafting, when it did occur, appeared to come primarily as a result of the invasion of bacteria carried upon the surface of seedling roots. It seemed possible to reduce the contamination by these bacteria in two ways: (a) washing from the seedlings all contaminating soil and treating them with antiseptic as developed by a number of writers, reviewed by Riker *et al.* (8), and (b) wrapping the graft with adhesive tape containing antiseptic. Neither of these methods, however, could provide, under commercial conditions, destruction of absolutely all the bacteria, because they may be harbored within certain openings, such as those left by small dead branch roots. On the other hand, both methods present advantageous commercial procedures in which perfection is not required. This question has been here approached from both of these standpoints.

[1] Published with the approval of the Director of the Wisconsin Agricultural Experiment Station. These studies were made in cooperation with Johnson and Johnson through the Crop Protection Institute, and with various members of the American Association of Nurserymen. This work was correlated with that done in cooperation between the Bureau of Plant Industry, U. S. Department of Agriculture and the University of Wisconsin.

The easier procedure, as far as the nurserymen are concerned, is the incorporation of an antiseptic within the adhesive tape, avoiding the use of antiseptic solutions. The incorporation of an antiseptic within the plaster mass presented several rigid requirements: (a) No interference with the adhensive quality of the tape; (b) strength enough to kill bacteria and mold without injury to the callus developed at the union; (c) strength enough to preserve the cloth of the tape for maximum protection without preventing the decomposition of the cloth and consequent girdling of the young apple tree.

The purpose of this paper is to describe the use of antiseptic solutions for cleansing the seedling roots prior to the grafting operation and to explain studies on the development of an antiseptic adhesive-tape wrapper.

The selection of an antiseptic was attended with certain difficulties. It seemed desirable first to make laboratory trials of the efficiency of the various antiseptics in common use by plant pathologists and others in relation to their toxic action on *Phytomonas rhizogenes* R. B. W. K. and S. This organism was selected because it is so much more important in the apple nursery than *Phytomonas tumefaciens* (Smith and Town.) Bergey *et al.* The difficulty involved in incorporating an antiseptic into the plaster mass of the adhesive tape made it seem important to approach the problem in two ways: (a) the treatment of artificially infested seedling apple roots with antiseptic solutions, and (b) the introduction of antiseptics into the plaster mass. In addition it appeared that the concentration of the antiseptic employed was of as great importance as the kind. It was not possible to include all kinds of antiseptics in the plaster mass because of certain chemical reactions that interfered with the adhesive qualities of the material, and because certain antiseptics were too volatile.

The basic principles involved in these studies are quite old. The foundation upon which the superstructure of modern surgery rests was stated by Sir Joseph Lister (5) and may be summarized as follows: Decomposition in a wound and affections due to decomposition are intimately connected with microorganisms coming from without. The wound treatment and dressing should prevent the access of microorganisms, and when these have entered, notwithstanding every care, they should be killed or rendered incapable of harm. The substances used for killing microorganisms should not irritate the wound at all, or at least, very little.

One of the writers (Kilmer) was impressed by the striking way in which these processes outlined for the practice of modern surgery have been applied to horticultural practice, as explained in detail in the following pages. While the nurseryman cannot in practice surround himself with the safeguards available to the surgeon, he can in some measure employ the same principles. The nurseryman can apply cleanliness and antiseptics and can avoid infectious particles.

The evolution of Listerism, as exhibited in modern surgery, has bestowed incalculable benefits upon mankind. While the methods herein outlined will not produce such heroic results, it is considered that their study, extension, and practical application will redound to the benefit of horticultural art.

ANTISEPTICS IN SOLUTION

The antiseptic solutions employed are considered first. Since it is well known that the action of an antiseptic against bacteria in a water solution may be greater than when in the presence of tissue, these trials were conducted on seedling apple roots under conditions simulating those found in the nursery. Seedling roots were washed free from surface soil and cut into pieces 3 to 4 inches long. These pieces were soaked from 2 to 4 hours in a suspension of the hairy-root bacteria. The roots were then submerged for varying periods (1, 5, and 10 minutes, respectively, in different tests) in the chemical solutions. After this treatment they were placed in sterile tubes. Doubtless longer treatment would have been more effective in certain cases. The roots were kept at approximately 12° C. for several days, and then small portions (about 3 mm^2) of the surface tissue of each root were removed, placed in Petri dishes, respectively, macerated, and flooded with agar. If any bacterial colonies developed, their characters were examined and their number recorded.

The concentrations of the antiseptic solutions employed were found to influence the results very considerably. Since the examination of the tissues was delayed for several days after their treatment, there was opportunity to determine whether the toxic action of the antiseptic had been injurious to the seedling root. When such injury did occur, it was conspicuous because of discoloration. The injury to the seedling roots was also checked by subsequent examination of their ability to produce callus tissue under favorable conditions. Silver nitrate and mercuric cyanide were, in some concentrations, very effective against the bacteria, but appeared to be injurious to the roots. Except for copper sulphate, none of the other chemicals, as employed, appeared to produce a harmful effect on the root tissue. Mercuric chloride solution, 1:1000, killed all the bacteria. The next in efficiency was cadmium chloride, 1:100. These tests were repeated from 2 to 20 times and a number of them are summarized in table 1.

Various other well-known antiseptics, such as formaldehyde and sodium hypochlorite, were omitted from the preliminary surveys because such volatile substances could not be incorporated satisfactorily into the plaster mass of the adhesive tape. The efficiency of the mercuric chloride, 1:1000, with which many of the nurserymen were familiar in connection with seed-potato treatment, made further study in this direction unnecessary.

TABLE 1.—*Summary of trials to determine the effect of various antiseptic solutions both on apple seedling roots, and on hairy-root bacteria on their surface*

Chemical	Concentration	Repetitions of tests[a]	Roots yielding bacteria	Killing of bacteria	Injury to roots
		No.	*No.*	*Per cent*	
Mercuric chloride	1:250	3	0	100	0
	1:400	3	0	100	0
	1:1000	20	0	100	0
	1:2000	3	8	47	0
Cadmium chloride	1:100	10	0	100	0
	1:200	10	7	86[b]	0
	1:400	9	6	87	0
	1:800	6	9	70	0
Ferric ammonium citrate	1:50	3	10	33	0
	1:100	9	40	11	0
	1:200	9	45	0	0
	1:400	8	39	2	0
Sodium salicylate	1:50	3	10	35	0
	1:100	6	28	7	0
	1:200	6	26	13	0
	1:400	3	15	0	0
Phenol	1:100	3	10	33	0
	1:200	6	25	16	0
	1:400	6	26	13	0
Organic mercury[c]	1:100	5	6	76	0
	1:200	3	4	73	0
	1:400	6	11	63	0
Silver nitrate	1:500	3	0	100	+
	1:1000	5	0	100	+
	1:2000	3	15	0	+
Thymol	Saturated	3	15	0	0
Hydrogen peroxide	Commercial	3	15	0	0
Mercuric cyanide	1:500	2	0	100	+
	1:1000	9	1	98	+
	1:2000	3	12	20	0
Copper sulphate	1:50	4	8	60	+
	1:100	4	12	40	+
	1:200	4	16	20	0
Bordeaux mixture	2:2:50	2	10	0	0
	4:4:50	2	10	0	0
	8:8:50	2	10	0	0
Hexyl-resorcinol	Undiluted	4	19	5	0
	1:1	4	19	5	0
	1:2	4	19	5	0
Control[d]		15	75	0	0

[a] Five roots were used in each test.

[b] In one of these tests, perhaps through error, the results gave a low figure. If this be disregarded, then the percentage of killing effect of bacteria would be 93 instead of 86.

[c] Hydroxymercurichlorophenol sulphate.

[d] Roots soaked in bacterial suspension and then dipped in water. The bacteria were covered in every case.

The results of field tests are reported in later pages.

Certain disadvantages appear in connection with the commercial use of antiseptic solutions on seedling roots such as: (a) Extra expense is involved for the labor, equipment and materials; (b) the seedling roots must be dried after treatment in such a manner that they are not injured; (c) the concentration of the antiseptic must be weak enough to avoid injury to the roots; (d) the strength of the solution must be maintained at a suitable point or it may do more harm than good by spreading the bacteria (8).

Parallel to these laboratory studies tests were made with certain adhesive tapes into which different antiseptics in various concentrations, respectively, were incorporated.

ANTISEPTICS IN ADHESIVE TAPE

The composition of the adhesive mass employed for spreading on muslin was similar to that in common use. The formula, as supplied by the manufacturer, was as follows: gum elastica, 30 per cent; resin from *Picea excelsa,* 15 per cent; resin from *Pinus australis,* 15 per cent; gum olibanum, 10 per cent; and wax, 5 per cent. The remainder was principally a vegetable filler, such as powdered orris root. Various antiseptics were worked into this material before it was spread on the cloth. In each case the concentration is given in percentage by weight.

Different antiseptics or insect repellents were included in the plaster mass in preliminary experiments and were mixed mechanically with the plaster at the time it was prepared. The character of this plaster mass is such that it cannot be mixed by hand, especially if a uniform product is desired. Among the chemicals included in the early tests were phenol, boric acid, thymol, salicylic acid, mercuric iodide, two organic mercury compounds, and nicotine.

BACTERICIDAL ACTION OF ANTISEPTIC TAPE

The antiseptic property of various tapes was tested in preliminary experiments in which the tape was placed in a sterile moist atmosphere and suspensions of the hairy-root organism were smeared over the surface. It became obvious in early experiments that the antiseptic in the adhesive tape was quite different in its action from that of the same antiseptic in solution. Since the plaster mass is relatively impervious to moisture, the effect of any antiseptic in the plaster mass was, naturally, very considerably reduced in intensity. In these preliminary trials it appeared that the bacteria were killed in 1 hour on the mercuric-iodide tape, in 3 hours on the salicylic-acid tape, in 2 days on phenol tape, and in 8 days on boric-acid and thymol tape. They were still alive on nurseryman's tape and on glass slides after 8 days. Since these preliminary tests involved relatively

large masses of bacterial slime, an effort was made to secure a more delicate test against a relatively smaller number of organisms.

Better indications of the toxic action of the antiseptic tape were secured by placing discs (about 8 mm. in diameter) of the antiseptic tape in the bottom of Petri dishes and pouring melted agar, seeded with hairy-root bacteria, over the surface. After a suitable incubation period the relative width of the clear zone about the antiseptic tape disc provided a more delicate index of the antiseptic value of this material. In the preliminary tests the average width in millimeters of the clear bacteria-free margins around the several discs of tape containing antiseptic were as follows: cresol (1:10), 6.5; mercuric iodide (1:100), 5.0; salicylic acid (1:5), 3.0; boric acid (1:10), 2:5 phenol (1:10), 2.0; organic mercury (1:1000), 1.5; thymol (1:20), 1.5; surgeon's 1.5; hydroxymercurichlorophenol sulphate (1:100), 1.2; hydroxymercurichlorophenol sulphate (1:500), 1.0; Graftex, 1.0; nicotine (1:100), 1.0; nurseryman's, 0.0. Although there was no clear area around the disc of nurseryman's tape, there was a small space about 4 mm. in diameter directly above the disc of tape that was apparently free from bacterial colonies. These tests were twice repeated in triplicate with similar results.

TABLE 2.—*Summary of experiments on the toxic effect of various antiseptic tapes upon the growth of hairy-root bacteria in Petri dishes containing bacterial suspensions of varying density*

Antiseptic in tape	Concentration of antiseptic	Margin of clear area around disc of tape in plates seeded with approximately			
		5000 bacteria	10,000 bacteria	20,000 bacteria	500,000 bacteria
		mm.	*mm.*	*mm.*	*mm.*
Mercuric chloride	1:100	36.75	35.50	30.50	10.25
	1:200	35.00	34.25	28.00	10.00
	1:400	29.75	25.25	17.00	9.50
	1:800	19.75	18.00	15.00	7.75
Cadmium chloride	1:100	7.50	6.50	5.50	3.50
	1:200	7.50	6.25	5.00	3.00
	1:400	6.00	5.25	4.00	2.50
	1:800	4.25	3.50	3.00	2.50
Phenol	1:100	3.75	3.00	2.00	2.75
	1:200	3.75	2.75	.....	2.50
	1:400	2.75	2.00	1.50	1.50
Salicylic acid	1:50	5.00	2.75	1.50	.....
	1:100	3.25	2.50	.....	.....
	1:200	3.00	2.25	1.50	.....
	1:400	2.25	2.00	1.00	.....

The concentration of the bacteria in relation to its effect on the width of the bacteria-free margin about the adhesive tape discs was tested. Tape containing mercuric chloride and cadmium chloride in various concentrations were employed in plants seeded with bacterial suspensions in varying amounts. The results of these tests are shown in table 2, where it appears quite conspicuously that the larger the number of bacteria, the smaller the width of the bacteria-free zone. Because of this factor, the concentration of the bacteria in the various comparative tests was made as uniform as possible.

Field tests also were made with these tapes. The results are omitted because of their volume.

In further tests, because of the promise they had given in several directions, only mercuric chloride and cadmium chloride were employed. These studies, run in triplicate and repeated three times, gave results that confirmed those already mentioned. From these results it appeared desirable to examine tapes containing these chemicals in various concentrations applicable to nursery practices.

The bacterial action of these antiseptic tapes was further tried by wrapping them on roots, stems, and grafts of apple plants that had been previously inoculated with heavy suspensions of the hairy-root bacteria. Isolations were made from the surface tissues at various intervals after the treatment. Some of these tests were made in the laboratory, while others were conducted in the greenhouse and the field. The results of the laboratory trials, in which isolations from heavily inoculated and wrapped surface tissues were made about 2 weeks after treatment, showed that mercuric chloride tapes 1:100, 1:200, 1:400, and 1:800 killed bacteria to the extent of 100, 87, 73, and 73 per cent, respectively; and cadmium chloride tapes 1:100, 1:200, 1:400, and 1:800, to the extent of 100, 87, 73, and 66 per cent, respectively. The results of further tests in the greenhouse and in the field, summarized in table 3, showed that the bacteria were killed by the antiseptic tape in a relatively short time after the treatment. Isolations were attempted after various times with negative results. Since the bacteria were killed upon the surface, it seemed desirable to determine whether or not the antiseptic action of the tape would have any influence within cuts made in the apple tissue resembling those produced in grafting.

The extension of antiseptic action from the antiseptic tape into cuts made in apple tissue was studied in experiments similar to those mentioned above except that the stems or roots were cut approximately halfway through before the application of the bacterial suspension. Grafts whose cut surfaces were treated with bacterial suspension before planting in the field were also used. Under such conditions it appeared more likely that the bacteria might be protected against bactericidal action from the anti-

TABLE 3.—*Summary of tests to determine the longevity of hairy-root bacteria smeared on the surface of roots or scions of apple grafts and covered with various antiseptic tapes*

Treatment		Roots or scions[a] from which hairy-root bacteria were recovered after				
		1 day	4 days	12 days	20 days	60 days
		No.	*No.*	*No.*	*No.*	*No.*
IN GREENHOUSE						
Bacteria applied						
Tape wrappers:						
Mercuric chloride	1:100	0	0	0	0	0
Do	1:200	0	0	0	0	0
Do	1:400	0	0	0	0	0
Cadmium chloride	1:100	1	0	0	0	0
Do	1:200	2	1	0	0	0
Nurseryman's tape		3	3	2	3	2
No wrapper		3	2	3	3	2
No bacteria applied						
No wrapper		0	0	0	0	0
IN FIELD						
Bacteria applied						
Tape wrappers:						
Mercuric chloride	1:100	.....	.....	0	0	0
Do	1:200	.....	.....	0	0	0
Do	1:400	.....	.....	0	0	0
Cadmium chloride	1:100	.....	.....	0	0	0
Do	1:200	.....	.....	0	0	0
Nurseryman's tape		.....	.....	2	2	2
String		.....	.....	3	3	3
No bacteria applied						
String wrapper		.....	.....	0	0	0
Nurseryman's tape wrapper		.....	.....	0	0	0

[a] Three employed in each test.

septic tape. On the other hand the spread of the antiseptic through the agar in the Petri-dish tests suggested that a certain amount of spread might take place also into the apple tissue. Since the greatest danger of infection in the apple graft is from the introduction of bacteria into the cortical tissue, it appeared that the antiseptic need not necessarily penetrate far into the wound in order to be relatively effective. The results of these tests are recorded in table 4. As was to be expected, the bactericidal action in the cut was very much slower than that on the surface of the apple tissue. However, it appeared after some time that the possibility of recovering the bacteria from such cuts was very greatly reduced. Further evidence along

TABLE 4.—*Summary of tests to determine the longevity of the hairy-root bacteria smeared into cuts made in apple roots and stems and in the unions of grafts wrapped with various adhesive tapes*

Treatment		Roots, stems, or grafts[a] from which hairy-root bacteria were recovered after				
		1 day	4 days	12 days	20 days	60 days
		No.	*No.*	*No.*	*No.*	*No.*
IN GREENHOUSE (cuts in roots and scions)						
Bacteria applied						
Tape wrappers:						
Mercuric chloride	1:100	3	2	2	0	0
Do	1:200	3	2	2	1	0
Do	1:400	3	3	3	2	0
Cadmium chloride	1:100	3	2	3	2	1
Do	1:200	3	3	3	2	1
Nurseryman's		3	3	3	3	3
No wrapper		3	3	3	3	3
No bacteria applied						
No wrapper		0	0	0	0	0
IN FIELD (graft unions)						
Bacterial applied						
Tape wrappers:						
Mercuric chloride	1:100	.....	.....	3	0	0
Do	1:200	.....	.....	3	1	0
Do	1:400	.....	.....	3	2	0
Cadmium chloride	1:100	.....	.....	3	1	1
Do	1:200	.....	.....	3	2	2
Nurseryman's		.....	.....	3	3	3
String		.....	.....	3	3	2
No bacteria applied						
String wrapper		.....	.....	0	0	0
Nurseryman's tape wrapper		.....	.....	0	0	0

[a] Three employed in each case.

this line is considered later in connection with the hairy-root developed on grafts inoculated and wrapped with antiseptic tape.

In the previous experiment the question arose as to whether the mercury was actually penetrating into the apple tissue, perhaps assisted by surface films of moisture. This question was approached experimentally by making analyses of cortical graft tissue that had been kept wrapped for a few weeks with tape containing mercuric chloride. The surface of the grafts was cleaned with ether to remove any of the plaster mass that adhered after the removal of the tape. These examinations were performed by Eugene Schoeffel under the direction of Professor K. P. Link, who found that by

using the method of Lamholt and Christiansen (3) he was able to secure traces of mercury from this cortical tissue. The amounts of mercury in 0.5 gm. of dried tissue were as follows: Wrapped with nurseryman's tape, 0.00 mg.; with 1:400 mercuric chloride tape, 0.39 mg.; with 1:200 mercuric chloride tape, 0.73 mg.; and with 1:100 mercuric chloride tape, 2.85 mg.

Stand of Grafts Wrapped with Antiseptic Tape

The effect of antiseptic tape upon the growth of callus tissue at the graft union was a matter of some concern, since it appeared (1) that the antiseptic action was strong against the bacteria, (2) that this action penetrated into cut surfaces, and (3) that the tissue apparently absorbed traces of mercury.

The growth of grafts wrapped with tape containing various antiseptics was tried in the greenhouse to see what influence this tape would have upon the stand secured.The results of these greenhouse tests indicated that the kind of antiseptic tape might influence very considerably the stand of grafts secured. While there was considerable range in results in the greenhouse tests with relatively high experimental error, still it was obvious that some of the tapes had sufficient promise to justify more extensive field experimentation.

Duplicate tests with several antiseptic tapes were made in the field (Table 5). Here it appears also that some of the tapes killed off the grafts, while others had promise from the standpoint of antiseptic action without deleterious effects upon the stands. Similar tests, involving about 25,000 trees wrapped with mercuric chloride or cadmium chloride tape, are considered later in connection with the prevention of graft knots. These experiments indicate quite clearly that while sufficient antiseptic may be included to kill off the grafts, the amount may be so adjusted as not to reduce the stand.

A number of factors are involved in this question of stand and wrapping with adhesive tape. Among the more important are the following: (1) The adhesive tape forms a relatively impervious coating over the cut surface of the graft union, which prevents the absorption of moisture by callus at this point. Under conditions of drought in the early spring, tape-wrapped grafts may not secure moisture so easily as those wrapped with string. This consequence occurs very rarely. (2) The mechanical action of the tape as a binder about the union prevents the formation of excess callus at the surface. The callus formation is forced between the cuts of the scion and the root, thus providing a more perfect union. (3) The tape prevents the entrance of contamination from the exterior after the graft is made and, particularly when an antiseptic is present, reduces the activity of molds, which sometimes may cause damage when other wrappers are employed.

TABLE 5.—*Summary of results taken in 1929 and 1930 of experiments that compare the effect of various antiseptic tapes upon the stand of apple grafts planted at various localities*

Location of experiment	Tape employed	Variety of apple graft used	Grafts wrapped	Stand
				Per cent
Missouri	Phenol	Rome Beauty	300	96
	Boric acid	do	300	67
	Mercuric iodide	do	300	79
	Sodium salicylate	do	300	23
	Nurseryman's	do	1000	92
Minnesota	Phenol	Wealthy	250	27
	Boric acid	do	250	26
	Mercuric iodide	do	250	90
	Sodium salicylate	do	250	8
Kansas	Mercuric iodide	Yellow Transparent	500	0
	Cresol	do	440	1
	Organic mercury[a] (1:100)	do	420	97
	do (1:500)	do	500	91
	Surgeon's	do	500	78
	Nurseryman's	do	500	93

[a] Hydroxymercurichlorophenol sulphate.

(4) The tape wrapper makes the union relatively solid, so that there is comparatively little chance of loss from breaking of grafts incident to manipulation in storage and planting operations. (5) If too many layers of tape, especially of antiseptic tape, encircle the graft at one point, the soil bacteria and fungi are unable to destroy the tensile strength of the cloth before the stems begin to enlarge in caliber, and girdling action may result. When the advantages are weighed against the disadvantages and the averages of a series of tests are taken, it appears, as Riker *et al.* (8) reported, that the ordinary nurseryman's tape does not change materially the percentage of stand secured from the grafts, although it does prevent the loss due to breakage in handling. From the foregoing report on stands, it appears that certain antiseptic tapes, when properly prepared, do not reduce the stand.

Preservation of Cloth by Antiseptics

The possibility of girdling action by the antiseptic tape was a matter of concern. Greenhouse tests were made with various antiseptic tapes, some of which decayed in a satisfactory length of time while others lasted too long to be safe as wrappers for apple grafts. The details of these results are omitted because of their volume. The more promising of the antiseptic tapes were tried in the field and their girdling action was recorded under

the most severe drought conditions. During the period 1930–1933 the greatest amount of girdling was found in small experimental plots in Wisconsin. Mercuric chloride tape 1:100 produced 18 per cent girdling, while mercuric chloride 1:200 produced 5 per cent girdling. Lower concentrations of mercuric chloride and the concentrations of cadmium chloride employed produced no girdling and none was observed with nurseryman's tape or with string wrappers. Because of the importance of this question of the duration of tape, records were taken of girdling action along with the records on the influence of different tapes upon the development of graft knots.

The decay of the adhesive tape after a suitable period should not be stressed so greatly as to minimize the importance of having it last long enough. Some of the varieties of tape that had been employed (8), have been unsatisfactory because the cloth disintegrated too quickly. It is altogether desirable that it should last until the maximum protection is provided in strength at the point of union and against the attack of various root-chewing insects. On the average, cloth that gives way in much less than 12 weeks or fails to give way in more than 15 weeks is relatively undesirable. This time varies considerably in different localities during the same season and in the same locality during different seasons. It may be observed that when the season is dry the apple grafts do not make such vigorous growth and the tape has a longer period of time in which to decay. On the other hand, when the season is wet, the grafts may grow more vigorously, but the tape decays more rapidly. The cloth may still appear to be undecayed when the action of soil fungi and bacteria have reduced its strength practically to zero. Consequently, the question of whether the cloth has decayed or not should be determined by an examination of its tensile strength rather than by its appearance.

CONTROL OF GRAFT KNOT

The controlling effect of various antiseptic tape wrappers was studied in a variety of situations. Certain preliminary studies on inoculated grafts were made in Wisconsin and the results are recorded in table 6. Even when a heavy suspension of pathogenic bacteria was introduced into the cut at grafting time, the antiseptic tape prevented disease development in many cases.

Experimental tests on ordinary commercial grafts were made in 1931, 1932, and 1933. These studies are recorded in table 7, which shows that, as previously reported for nurseryman's tape (8), variations were noted depending upon local conditions. Where no diseases appeared, little control was provided. However, when the infectious bacteria were carried upon the seedling roots, very promising control was secured. It will be observed

TABLE 6.—*Summary of results after one growing season showing the effect of various wrappers on the prevention of hairy root at the unions of piece-root Wealthy-apple grafts, inoculated when grafted*

Treatment	Trees examined	Trees with hairy roots	Healthy trees	Girdled trees
Inoculated when grafted	*Per cent*	*Per cent*	*Per cent*	
Tape wrapper:				
Mercuric chloride 1:100	78	44	56	18
Do 1:200	98	44	56	5
Do 1:400	42	62	38	0
Cadmium chloride 1:100	50	86	14	0
Do 1:200	45	89	11	0
Nurseryman's	119	97	3	0
String wrapper	54	100	0	0
Not inoculated				
Nurseryman's tape wrapper	36	11	89	0
String wrapper	109	3	97	0

that while the results with one strength of antiseptic tape were favorable in one place, the results with another were, the same year, more favorable in a different place. Variations occurred in the same nursery in different years. On the average, it appears that the antiseptic tape containing mercuric chloride was superior to any of the others and that a balance was desirable between the extremes of concentration employed with this antiseptic. From these tests it appeared to the writers that a concentration of mercuric chloride of 1:300 was strong enough to provide the antiseptic action desired, and to preserve the cloth for the desired period. At the same time it was weak enough not to injure the graft union and not to preserve the cloth from disintegration beyond the desired period for protection. Consequently, for the last year's tests, this concentration only was used and was found to be satisfactory under the variety of conditions employed. In these tests, very little disease appeared in the controls. It is obvious, however, that the control measures applied in the form of antiseptic tape could have no influence upon factors that induce the development of hairy root during the latter part of the first year or during later years; except, perhaps, a slight action by the residual plaster mass in repelling chewing insects. In a very few nurseries where the infection of the seedling roots was particularly heavy at the time of grafting, the control of graft knots at the union, while a considerable improvement over that secured with nurseryman's tape, was still not fully satisfactory.

In such cases tests were made with treatments of seedling roots in mercuric chloride solution, 1:1000, which is discussed in an earlier section of this paper, about a week before making the graft. The grafts were

TABLE 7.—*Summary of results from experiments during 1931, 1932, and 1933 on the prevention of graft knots by various wrappers*

Wrapper	Trees examined	Stand	Trees smooth	Trees girdled
Results after one season (1931)[a]	*No.*	*Per cent*	*Per cent*	*Per cent*
String	1000	69	76	0
Nurseryman's tape	900	66	86	0
Cadmium chloride tape 1:400	900	69	89	0
Mercuric chloride tape 1:400	900	67	93	0
Results after two seasons (1931 and 1932)[b]				
String	1355	72	53	0
Nurseryman's tape	1590	67	67	0
Cadmium chloride tape 1:400	1513	69	70	0
Mercuric chloride tape 1:400	1355	67	78	0
Results after one season (1932)[c]				
Mercuric chloride tape 1:100	585	74	94	5
Do 1:200	600	74	94	2
Do 1:400	585	72	94	0
Cadmium chloride tape 1:100	580	62	92	0
Do 1:200	555	71	90	0
Nurseryman's tape	585	78	84	0
Results after two seasons (1932 and 1933)[d]				
Mercuric chloride tape 1:000	1093	70	86	1
Do 1:200	1081	69	86	0
Do 1:400	1233	67	86	0
Cadmium chloride tape 1:100	933	53	81	0
Do 1:200	810	64	81	0
Nurseryman's tape	1249	73	80	0
Results after one season (1933)[e]				
Mercuric chloride tape 1:300	1243	84	97	0
Nurseryman's tape	661	83	95	0

[a] Summary of 4 parallel tests in 4 nurseries in 4 states.
[b] Summary of 3 parellel tests in 3 nurseries in 3 states.
[c] Summary of 7 parallel tests in 6 nurseries in 6 states.
[d] Summary of 5 parallel tests in 5 nurseries in 5 states.
[e] Summary of 6 parallel tests in 3 nurseries in 3 states.

wrapped with nurseryman's tape. The results summarized in table 8 show that such treatment, while it may reduce the stand slightly, aids considerably in reducing graft knots.

The treatment of the seedling roots with mercuric chloride solution, subsequently wrapping them with the antiseptic tape, is being continued, but the results are not yet available. The chief concern about this double treatment relates to stand. This should be a promising method in the relatively few places where hairy root is not controlled by the antiseptic tape alone.

TABLE 8.—*Summary of results taken in 1932 and 1933 on the effect of treating seedling roots with an antiseptic solution before grafting*[a]

Treatment	Trees examined	Stand	Trees smooth
Results after two seasons[b]	*No.*	*Per cent*	*Per cent*
Mercuric chloride, 1:1000	2126	59	93
None	2390	63	65
Results after three seasons[c]			
Mercuric chloride, 1:1000	620	63	81
None	1365	68	72

[a] Grafts made with both treated and untreated roots were wrapped with nurseryman's tape.

[b] Summary of 8 tests in 3 nurseries in 3 states.

[c] Summary of 2 tests in 2 nurseries in 2 states.

The recommendations under average conditions for the control of both infectious and noninfectious malformations at the unions of piece-root apple grafts are as follows: (1) Make the grafts from clean seedling roots that have no water on the surface at the time of grafting, and (2) wrap with antiseptic tape that meets the requirements previously outlined. The reasons for having the surface of the seedling dry are (1) to reduce the introduction of infectious bacteria at the time the cut is made and (2) because the adhesive tape does not adhere to a moist surface. No difficulty has been experienced with excessive desiccation of the seedling roots if they are not permitted to remain in the grafting room an unusually long time. In the relatively few nurseries where difficulty was experienced with hairy root due to heavy infection of the seedlings at the time of grafting, good results have been secured by washing the roots; by treating them in mercuric chloride, 1:1000; and by storing them for a week in clean packing material before grafting and wrapping with nurseryman's tape. The infections that occur during the latter part of the first season and during later seasons in the nursery come primarily following attacks by root-chewing insects or mechanical injuries with cultivators (2), (8), which open the way for infectious bacteria to enter. Banfield (1), Levine (4), and Patel (6) have reported that crown-gall bacteria may persist in the soil for several years. Knots that appear during this time may be reduced by planting the grafts in soil where susceptible crops have not been grown for 4 years, by planting in soil previously manipulated so as to be relatively free from root-chewing insects, and by suitable precautions against mechanical injuries near the crown during cultivation.

SUMMARY

Work already described (Riker *et al.*, 8), on the control of knots at the unions of nursery apple trees grown from piece-root grafts, has been con-

tinued and extended. Attention has been directed primarily against bacteria carried on the apple seedlings—a common source of inoculum.

Various concentrations of twelve antiseptic solutions were employed against hairy-root bacteria on the surface of apple seedlings. Mercuric chloride, 1 to 1000, and cadmium chloride, 1 to 100, were effective in killing all the surface bacteria without visible evidence of root injury.

Several series of antiseptics were incorporated into the plaster masses of the nurseryman's-tape wrappers. Their efficiency was tested against the bacteria in Petri dishes, on seedling roots, in cuts in seedling roots, and in grafts. Various trials were made in the laboratory, greenhouse, and field. The more promising antiseptics were employed in various concentrations for their effect on the bacteria, on the preservation of the cloth, on the stand, and on the prevention of graft knots. Mercuric chloride was the best antiseptic employed. Its concentration of 1 to 300 by weight was the most satisfactory.

Under ordinary conditions commercial control was secured merely by wrapping the graft unions with this antiseptic tape. It appeared superior to ordinary nurseryman's tape.

Under uncommonly severe conditions the best control was secured as follows: (1) A week before grafting the cleaned seedling apple roots were treated for a minute in mercuric chloride solution, 1 to 1000. (2) After grafting, the unions were wrapped with nurseryman's tape. The effect of wrapping antiseptic tape on grafts made with treated roots has not been determined.

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A STILBUM DISEASE OF FIG IN LOUISIANA

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INTRODUCTION

The fig, *Ficus carica,* is one of the few fruits that grow well in the southern part of Louisiana. It is not only the most widely grown fruit in that section of the State, but is probably enjoyed by the greatest number of people, as well. Most of the residents along the lower portion of the Mississippi River, Bayou Tèche, Bayou Lafourche, and other smaller streams have one or more fig trees in the yard, garden, or orchard. While a number of different fig varieties are grown to some extent, the Celeste is the one most commonly used. Many of the fig trees are grown in yards more or less shaded by large pecan, oak, or other trees or by buildings of various sorts. The fig trees growing in these damp, shady places are more susceptible to the attack of certain fungus parasites favored by such conditions. This paper deals with a new fig disease recently observed in this territory.

Diseased fig branches were sent in to the Department of Botany, Louisiana State University, in May, 1932, from Hohensolm (Ascension Parish), Louisiana. These branches were covered with small, round, pink heads borne on short stalks, the heads being composed of small, oval, unicellular hyaline spores. The fungus was obtained in culture quite readily by means of spore dilutions in string-bean agar. Preliminary inoculation tests made on young fig branches (Celeste variety) showed the fungus to be an active parasite. Young, infected fig branches were sent to C. L. Shear, Washington, D. C., who tentatively identified the fungus as *Stilbum cinnabarinum* (Mont.).

While a number of Stilbum species have been described from various sections of the United States, as far as the writer can find out from the available literature, *S. cinnabarinum* has not previously been found in this country. Members of the genus, Stilbum, are widespread over the tropics, in some cases causing destructive diseases, such as the "American coffee leaf disease" and others. Apparently, no Stilbum has been previously reported on species of Ficus.

REVIEW OF LITERATURE

A review of the literature on diseases caused by Stilbum species indicates that there are 2 entirely different fungi that have been called Stilbum by various writers.

According to Ashby (2), Cooke (14), in 1880, described the reproductive bodies of a fungus associated with a leaf and berry spot of coffee, which he

called *Stilbum flavidum*. Some years later (1899), Massee (23) mentioned the coffee disease in Nicaragua caused by *S. flavidum* (Cke.).

In 1903, Kohl (20) proposed that the name of the coffee leaf-disease fungus be changed from *Stilbum flavidum* (Cke.) to *Stilbella flavida* (Kohl). Since that time numerous other investigators have noted a similar coffee disease, which they attributed to either *Stilbella flavida* (Kohl) or *Stilbum flavidum* (Cke.). Among these workers, may be noted Puttemans (30) (1904), Delacroix (17) (1911), Fawcett (18) in 1910 and later, in 1915 (19), Maublanc and Rangel (25) (1913), McClelland (27) (1921), Cook (13) (1926), Ciferri (12) (1926) in the Dominican Republic, Chardon (11), in 1927, in Colombia, and Abbott (1), in 1931, in Peru. In 1913 (25) and later, in 1914 (26), Maublanc and Rangel mentioned the existence of a perfect stage of *Stilbum flavidum,* which they called *Omphalia flavida* (Maubl. & Rangl.). In 1925, Ashby (2) described the reproductive bodies of *S. flavidum* as 2–2.5 mm. in height with heads 0.25–0.33 mm. in diameter. He continued "Many Stilbum heads from pure culture and from leaf and berry spots heve been examined for sporulation, but no spores have been found; the enlarged peripheral cells have been seen to give rise only to hyphae in water and on culture media." Ashby clearly demonstrated the presence of the Basidiomycete stage (*Omphalia flavida*) of *Stilbum flavidum* which had previously been mentioned by Maublanc and Rangel.

In 1907, Bernard (8) described a new fungus disease of tea caused by *Stilbella theae* Bernard. He distinguished this fungus from *Stilbum nanum* by the presence of conidia on the stalked heads. Also, in 1920, Averna-Sacca (5) described a species of Stilbum on cacao, with synnemata bearing spherical heads made up of elliptical, hyaline spores. Averna-Sacca (4) also mentioned *Stilbum nanum* (Massee) variety *gossypina* (Sacca), a similar fungus with spherical spore heads, on cotton. Later, the same author (7), in 1927, described a Stilbum causing a discoloration and rot of roses, the synnemata of the fungus having orange or dark red heads and bearing elliptical, hyaline, thin-wall spores. Staner (35) also described a Stilbum on stems of *Vanilla planifolia,* which had small conidia borne in heads, on reddish stalks. In 1925, Jenkins[1] described a similar Stilbum on rose twigs from Cuba, probably the same fungus as that noted by Rorer (31) from Trinidad. Petch (29) noted the common occurrence of *Stilbum cinnabarinum* on dead Hevea bark in Ceylon. It is evident that this group of Stilbums is entirely different from *S. flavidum.*

Other writers have observed diseases caused by Stilbum species, but many of them did not make their descriptions complete enough to deter-

[1] Jenkins, A. E. (*Stilbum* sp. on rose from Cuba). In Martin G. H. Diseases of forest and shade trees, ornamental and miscellaneous plants in the United States in 1924. U. S. Dept. Agr. Bur. Plant Ind. Plant Dis. Rptr. Suppl. 42: 363. 1925. (Mimeogr.)

mine in which group they belong. In 1898, Massee (24) mentioned *Stilbum nanum* on tea; followed by Delacroix (16) in 1902, Watt and Mann (36) in 1903, and Butler (10) in 1905. Numerous others in later years have mentioned this fungus as being destructive on tea plants. In 1931, Leach (21) mentioned a die-back of tea due to *Stilbella* sp., closely related to *Stilbella theae*. Blanchard (9), in 1928, mentioned *Stilbum* sp. on yerba maté (*Ilex paraguensis*) and Averna-Sacca (3) in 1919 observed *Stilbum* sp. causing a serious disease of pear in Brazil. More recently (1931), Mason (22) described an entomogenous fungus, *Stilbella kervillei* Lindau in England. He noted that the fungus is characterized by synnemata up to 5 mm. in height with yellow, waxy heads 4 mm. in height and 0.36 mm. broad, and branched conidiophores $20 \times 1\ \mu$, with small hyaline conidia of 2 types.

Another paper by Averna-Sacca (6) further complicates the Stilbum problem. According to Ashby (2) he mentions "A leaf, berry, and twig spot disease of coffee in São Paulo, Brazil, associated with the conidial synnemata of a Stilbella having spherical, vermilion spore heads. Although perithecia were not seen, he referred this fungus to *Sphaerostilbe flavida* Mass., citing *Stilbum flavidum* Cke., *Stilbella flavida* Kohl., and *Omphalia flavida* Maubl. and Rangl. as synonyms." Ashby writes "it is evident that the conidial fruiting body described by him (Averna-Sacca) had nothing in common with the Stilbum body of the American coffee leaf disease, and it is not clear from his account that he was dealing even in part with that disease." There are evidently 2 tropical coffee diseases, caused by 2 fungi that are similar in appearance, but quite different when examined closely.

The original description of Stilbum (Tode) as given by Saccardo (33) is as follows: "Stroma tereliusculum apice capitato-conidiophorum ex hypis coalitis conflotum. Conidia minuta continua muco primitus abvoluta." This description indicates quite clearly that there are conidia associated with the fungous heads, which necessarily eliminate the so-called "Stilbum" forms that do not have conidial heads.

Apparently, there have been 2 different organisms called by various writers ether *Stilbum flavidum* (Cke.) or *Stilbella flavida* (Kohl.). They are (1) a true Stilbum, characterized by the production of short synnemata, with small spherical heads composed of large numbers of small, ovate, unicellular conidia, and the (2) "pseudo-Stilbum," originally described as *Stilbum flavidum* (Cke.), which has small spherical heads produced on short stalks very similar in appearance to those of the true Stilbum, but the heads have no conidia associated with them.

The fungus associated with the fig disease in Louisiana is a true Stilbum having the conidial heads characteristic of that genus.

THE FIG DISEASE IN LOUISIANA

Symptoms.—The first evidence of the Stilbum disease on fig is the presence of dead twigs scattered about over the tree. Usually the very youngest twigs are killed back. The small pink fungus heads (Fig. 1, A and B) appear in large numbers on the bark of the dead or dying branches. There may be a definite canker stage when larger branches are affected and sunken areas may appear in large branches at the base of smaller ones. The fungus usually develops much more slowly in the larger branches than in the small twigs. The tissues just beneath the bark of diseased twigs turn a dark brown and this discoloration usually extends from 3 to 10 cm. in advance of the fungus heads, and extends through the wood into the pith. Small boring insects often infest the twigs soon after they are killed and make the destruction complete. Such diseased branches also may be severely affected by winter injury. The fungus may spread into the larger branches and eventually destroy the entire tree, or make it worthless, depending upon whether or not conditions are favorable for its development. On the other hand, many old fig trees have been observed that had a number of dead branches covered with Stilbum heads, but apparently the fungus was spreading very slowly, and causing very slight damage. No Stilbum infection has been observed on either fig leaves or fruit.

Distribution and Importance.—The Stilbum disease has been found scattered over most of the southern part of the State, from Baton Rouge as far west as Lafayette and north to Bunkie. One small infection also was found near Gulfport, Mississippi.

The importance of the disease is rather difficult to estimate. Many fig trees are grown for ornamental purposes as well as for the production of fruit. Any condition that causes the death of a few branches and results in such trees looking ragged and unhealthy is of some importance. Some trees are killed outright and others are so badly damaged that they must be destroyed, while many trees have only a few infected twigs and are not seriously damaged by the fungus.

Most of the damage observed on fig trees in the southwestern section of Louisiana has been found on the Celeste variety. One infection was found on a planting of Magnolia fig trees, but the damage was much less severe on trees of this variety. In one orchard where trees of the Magnolia variety were found surrounded by Stilbum-infected Celeste trees, the former have remained entirely free of Stilbum infection. Since the Celeste is the best quality fig, as well as the most widely grown variety in this section, the disease is of considerable economic importance. It can be safely stated that the Stilbum disease is second in importance to thread blight caused by *Corticium koleroga* (Burt.), among fig diseases in this section of the State.

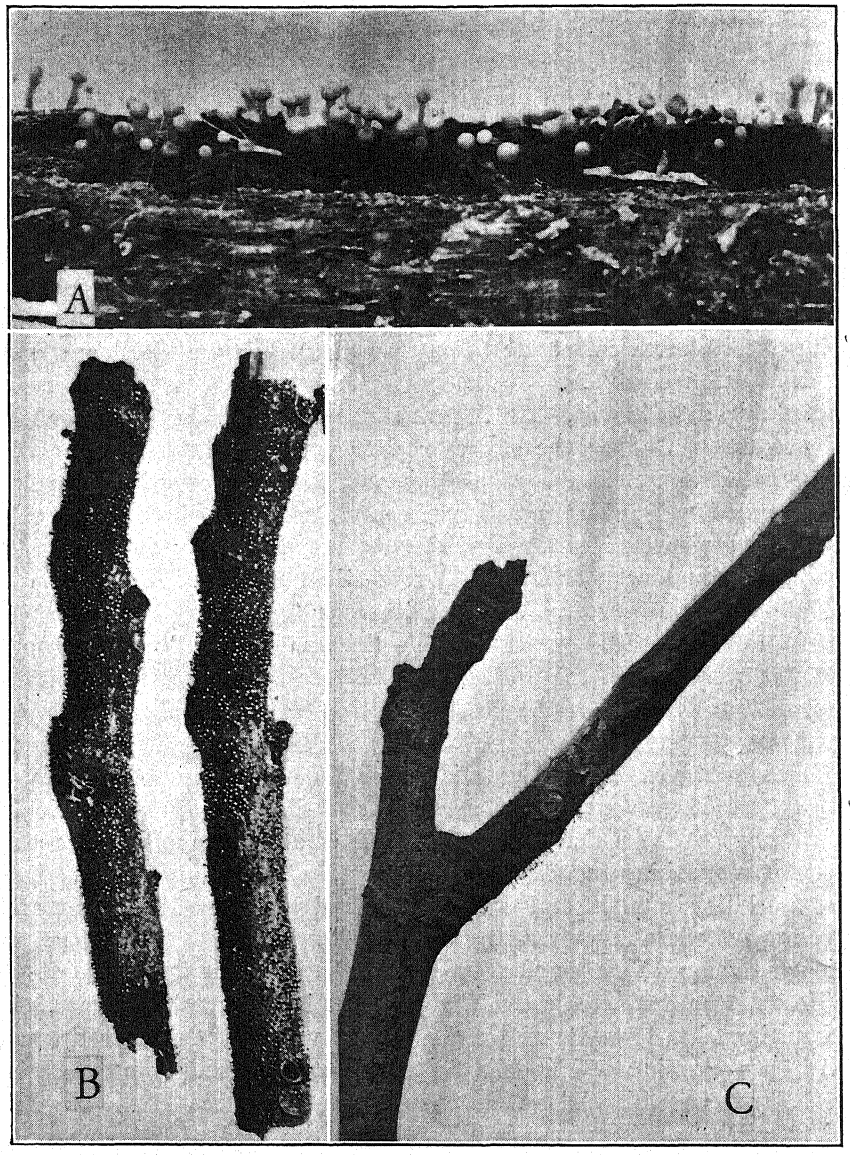


FIG. 1. *Stilbum cinnabarinum* on fig branches. A. Stilbum heads on an old twig. ×6. B. Naturally infected twigs showing numerous spore heads. Slightly reduced. C. An inoculated twig showing spore heads around point of inoculation. Photograph taken 38 days after inoculation. Natural size.

Environmental Conditions.—It is evident that infection takes place more readily under humid conditions. As was previously mentioned, the Stilbum disease is confined principally to very damp, shady places. Where it has been found in most serious form, the infected fig trees were usually growing in close proximity to large pecan or oak trees, or partly in the shade of certain buildings. But in some cases where inoculations were made on fig trees in the orchard away from any shade trees infection spread to the larger limbs quite rapidly.

Seasonal Development.—Observations made over a period of 2 years indicate that the Stilbum disease develops most rapidly during the late spring and summer, being much more severe during very wet spells. However, some inoculated trees have shown fungus development to some extent during the fall and through most of the winter. Temperature studies have shown that the fungus is favored by warm weather, although it grows slowly at fairly low temperatures.

Source of Infection.—Although the conidia are held firmly in the Stilbum spore heads, they become loosened somewhat when the heads come in contact with moisture. It is probable that wind, splashing rain, and possibly various insects may serve as disseminating agents for the conidia. The results of experimental inoculations indicate that some sort of wound is necessary before infection can take place. Since most of the infections seem to occur at or near the tips of the young twigs, it seemed probable that the fungus might enter through leaf and fruit scars. This theory was verified by a series of inoculations described later in this paper. Infections probably occur also through wounds made by boring insects, as well as through other types of wounds. During the summer of 1933 a number of Stilbum infections were found following wounds made by very large hailstones that fell during a severe storm in May.

The Causal Organism.—The first cultures of *Stilbum cinnabarinum* were obtained by making spore dilutions in string-bean agar from one of the small Stilbum heads. Transfers were made to sterile string-bean pods after 3 days. Growth was rapid and the first inoculations were made with spores from these bean-pod cultures. After the fungus had proved to be parasitic, single-spore cultures were obtained and all later inoculations were made with these.

On nearly all the culture media used this fungus produced single-cell conidia that were exceedingly variable in size (Fig. 2, B). The size varied from $3 \times 1.5\ \mu$ to $15 \times 5\ \mu$. The greatest variation in size of conidia occurred on acid-agar plates. When grown on sterilized fig twigs in large test tubes, some large, dark brown masses of irregular conidia were produced. The spores from these masses—probably sporodochial in character—were quite uniform in size and shape, and typical of those that develop

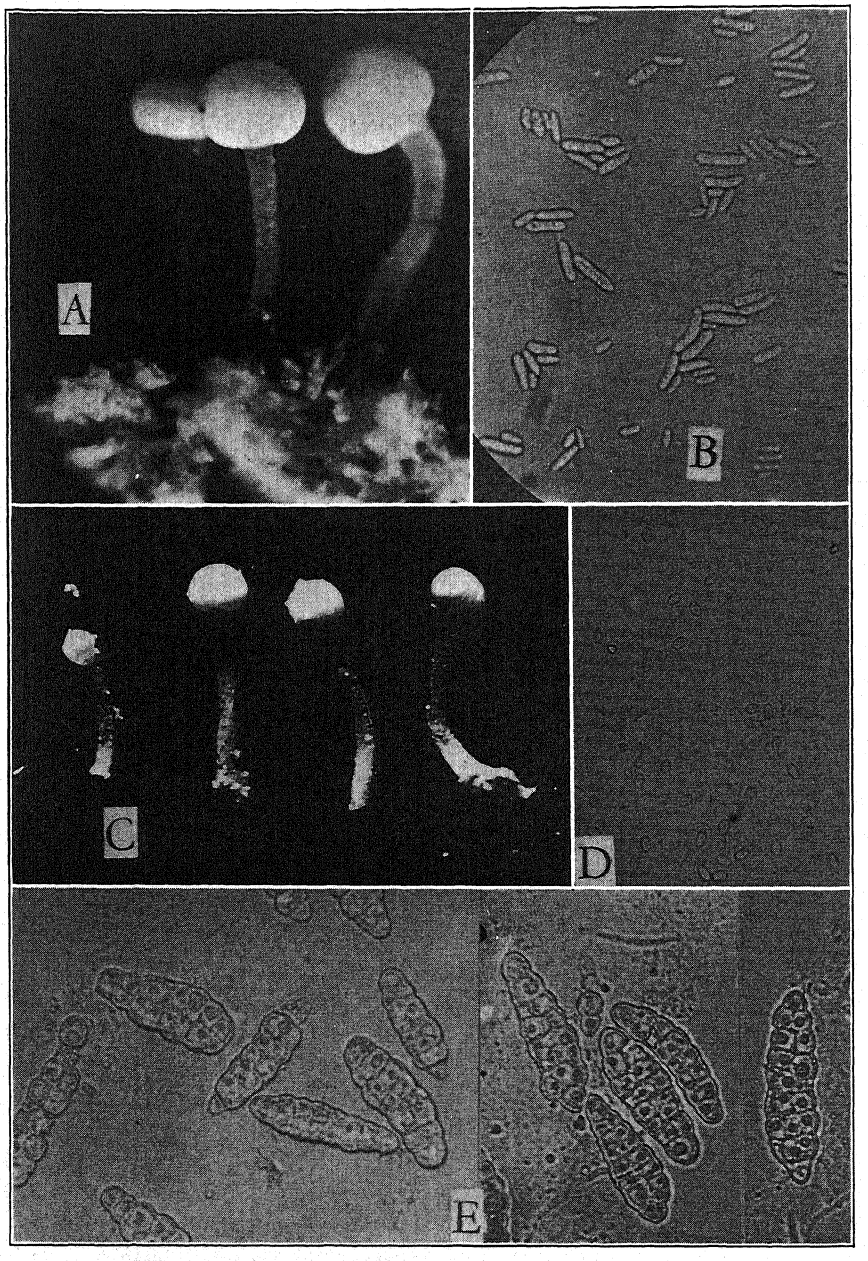


FIG. 2. Stilbum spore heads, conidia and ascospores. A. Stilbum heads showing typical condition. ×30. B. Conidia from pure culture (acidified string-bean agar) showing variation in size. ×386. C. Stalked heads similar to those in A. ×21. D. Stilbum conidia from spore head on infected branch. Typical of those always found under natural conditions. ×386. E. Ascospores of *Megalonectria pseudotrichia*. ×600.

on the stalked heads under natural conditions, the average size of these conidia being 5.7×3.0 μ. Although the fungus has been grown on a large number of different media and under varying conditions of light and temperature, only in a few cases have the stalked heads been produced in culture, and in most cases these did not develop normally.

One strain of the fungus obtained from a germinating ascospore produced the typical Stilbum heads rather consistently when grown on sterile string-bean pods, but they were smaller and more irregular in size and shape than those occurring naturally on fig branches. The conidia produced on these stalked heads were, however, quite uniform in size and shape.

On infected fig branches the Stilbum develops in a different manner from the way it grows in pure culture. The small pink heads break through the epidermis of the twig and the stalks gradually elongate until they reach their full length. The stalks—synnemata—develop either singly or in groups of as many as 4 or 5. Often 2 stalks may be joined together at the top in a single large head. The synnemata are smooth and vary from 50 to 200 μ in diameter and from 1 to 2 mm. in length, tapering slightly toward the upper end, each stalk being composed of numerous septate hyphae, compressed into a very compact mass that flares out somewhat at the top (Fig. 2, A and C). The heads, which are nearly spherical but slightly flattened at the base, are brick-red when first developed. Later the color may fade to a light pink, as the result of weathering. The heads range from 200 to 400 μ in diameter, the average being about 300 μ. The ovate, hyaline, single-cell conidia form a solid, compact mass on the top of the stalk, being held together by a genatinous material. The measurements made of several hundred conidia from different sources showed the size to vary from 3.5 to 7.5×2.5 to 4.1 μ, the average being about 6×3 μ (Fig. 2, D).

The above description of this fungus fits in fairly well with the original description of *Stilbum cinnabarinum* by Montagne (28) as given by Saccardo (34). The Louisiana form has synnemata somewhat shorter (1–2 mm.) than those described by Montagne (2–4 mm.) and conidia slightly larger, $6 \mu \times 3 \mu$ as compared with $5–6 \times 2$ μ. Since these differences seem to be only minor ones, the fungus herein described is considered to be synonymous with *Stilbum cinnabarinum* (Mont.).

Ascigerous Stage of the Fungus.—A few perithecia containing muriform, multiseptate ascospores were found associated with the Stilbum on fig branches about February 1, 1933. Single-ascospore cultures obtained from this material developed the characteristic Stilbum-type conidia in culture, and inoculations with these conidia into young fig branches produced the disease in typical form.

Early in January, 1934, a number of fig branches containing the bright red perithecia were found on Stilbum-infected fig trees in Ascension parish, some 35 miles below Baton Rouge. The perithecia were produced on the lower surface of dead twigs ½ to ¾ inch in diameter, singly or in groups of 2 to 5, often at the base of the old conidial stalks. They were often found attached to the bases of these old stalks. The perithecia when collected were bright red, almost perfectly round, quite soft in texture, and borne on very short stalks. In the moist, fresh condition they measured from 475 to 525 μ in diameter. Older perithecia, which had turned dark brown to almost black, were obtained from other trees. These measured from 300 to 450 μ in diameter and were somewhat sunken at the top. Soon after the round, bright red perithecia were collected (January 15), they contained a few asci with mature ascospores, and after being kept quite moist for a few days, they had considerable numbers of asci with mature spores. There were apparently no paraphyses present. The normal asci (Fig. 2, E), which contained 8 spores, were from 80 to 130 μ in length, and 16 to 20 μ in diameter, averaging 100 × 17 μ.

The ascospores were found to be very variable in size, ranging from 20 to 40 μ × 9–14 μ. Some ascospores were obtained from normal perithecia that were as long as 52 μ, but these had apparently begun to swell preparatory to germination. The ascospores were hyaline, muriform, with 5–7 transverse septa.

The asci could be forced out of the mature perithecia quite readily when slight pressure was exerted upon them, but the ascus wall itself was difficult to break. Often the spores were completely crushed before the ascus would break open, and the spores often germinated while still within the ascus. The ascospores germinated quite readily in tap water or in string-bean agar, producing numerous small germ tubes. As many as 25 germ tubes were counted on a single germinating ascospore.

Although *Stilbum cinnabarinum* has been grown on a large number of different culture media, including fig agar, and sterilized fig branches, as well as under varying light and temperature conditions, no perithecia have been produced in pure culture. However, perithecia have developed on fig branches inoculated with pure cultures of the fungus the previous year. All the observations made by the writer indicate that the perithecial stage of this fungus develops only in southern Louisiana under the most favorable moisture conditions, and then for a very limited period during January and February.

The perithecial stage of *Stilbum cinnabarinum* has been mentioned by Petch (29) as *Megalonectria pseudotrichia* (Schw.) Speg. The perithecia were said to occur along with the Stilbum heads on dead Hevea bark in Ceylon. The perithecial stage of the Louisiana Stilbum resembles

Megalonectria pseudotrichia (Schw.) Speg., as described by Saccardo (32), quite closely in most respects. However, the closely related form *Megalonectria nigrescens* (Kalch. & C.) Sacc. or *Sphaerostilbe nigrescens* (Kalch. & C.), as described by Cooke (15), also resembles the Louisiana form. Since the perithecial stage of the *Stilbum cinnabarinum* has been previously described by Petch as *Megalonectria pseudotrichia* (Schw.) Speg., the Louisiana form is tentatively listed under the same name.

Temperature Relations.—Two Stilbum cultures were used in temperature studies: (1) the original isolation, which had been used in most of the inoculation tests, and (2) a new culture isolated from another source in the fall of 1933. Both of these were monosporous cultures, known to be actively parasitic on fig branches.

Petri dishes of equal size, containing equivalent amounts of agar (string-bean and dextrose (Difco) agar), were inoculated in the center with small square pieces of agar from a rapidly growing culture of the fungus. They were kept at room temperature until the fungus started growth and were then placed in a series of incubators ranging from 6 to 37° C. Measurements were made of the diameter of the colonies (5 plates of each culture at each temperature) at 24-hour intervals, until the colonies covered the plates at the optimum temperatures. Growth was quite similar on both media with both cultures. There was some growth over the entire range of temperatures but very little at the extremes. The average growth in diameter of the colonies in mm. per 24-hour period was taken as a criterion of comparison. Growth was quite slow at 6° and 37°, the optimum temperature for most rapid growth being 28–30°, while the growth curve falls very rapidly between 32° and 37° C.

Pathogenicity.—The preliminary inoculation tests were made on fig trees of the Celeste variety in the vicinity of Baton Rouge, in June, 1932. Young, rapidly growing branches of the current year's growth were inoculated by making a small slit in the bark, inserting a small portion of a string-bean pod culture of the Stilbum into the wound, and sealing the slit with melted paraffin. A corresponding number of twigs were wounded and pieces of sterile bean pod inserted into the slits as controls. When examined 22 days later, 6 of the 10 inoculated twigs had some of the Stilbum heads developing on the bark around the point of inoculation. Two weeks later, several of the twigs were dead above the wound and the fungus had developed over the surface of the dead twigs. The controls all remained healthy.

The fungus was reisolated from these inoculated twigs and another set of inoculations made with the newly isolated cultures on August 2 of the same year. After 36 days, 6 of the 15 inoculated twigs were dead and the other 9 showed reddening of the bark, and some fungus growth around the point of inoculation. Later all of this lot of twigs were killed outright.

An inoculated twig with spore heads of the fungus is shown in figure 1, C. On February 17, 1933 (about 6 months after inoculation), the fungus had spread on 2 of the twigs to a point about 2 feet from the inoculation so as to kill back the 2-year-old wood for several inches. About a year later (18 months after inoculation), 2 main branches, 2 inches or more in diameter, had been killed back to the main trunk. This series of inoculations, along with several other more extensive ones, indicates that this Stilbum may be quite destructive under certain conditions.

In September, 1932, another set of inoculations was made on trees of the Celeste variety to determine more definitely how infection may take place. A known parasitic culture of Stilbum growing on sterile bean pods was used for inoculum. There were 4 sets of inoculations with appropriate controls, each consisting of 10 twigs: Lot 1 was inoculated in the usual way by means of a small slit in the bark of young twigs of the current year's growth; Lot 2, 2 leaves were broken off near the tip of each small twig and small portions of inoculum placed on the scars left after the removal of the leaves; Lot 3, 2-year-old wood inoculated by wounding in the usual way; and Lot 4, 2-year-old wood inoculated on the unwounded surface. Thirteen days later in Lot 1, one twig was dead, 3 others had Stilbum heads developed around the wound; while in Lot 2, 9 of the 10 twigs had Stilbum heads developing at the point of inoculation; 2 of the twigs in Lot 3 showed infection, while all those in Lot 4 remained healthy. When next examined on November 8 (60 days after inoculation), 6 twigs of Lot 1 were killed back 6–10 cm.; 7 of Lot 2 killed back 3–7 cm.; 6 of Lot 3 showed dark discolorations 3–5 cm. around the point of inoculation; while all the branches in Lot 4 (unwounded) remained free of infection.

During the following year many of these infections spread into the older branches, killing them back from 1 to as far as 3 feet in some cases, while in other twigs the spread was very limited. Two of the larger inoculated branches were killed back to the main trunk, and the dead wood was covered with the bright colored Stilbum heads. This test showed that the Stilbum does not penetrate the unbroken bark of the fig branch very readily, if at all, and that infection may take place through the leaf scars if the leaves are removed just before they are ready to shed. It seems likely that most of the natural infection occurring in fig trees may take place through the leaf or fruit scars near the tips of the small twigs. If favorable conditions occur, the infection may spread rapidly into the older branches.

No Stilbum infection has been found on either the leaves or fruit of the fig. Preliminary inoculation tests made on both the green fruit and on leaves have failed to produce infection. It is likely that the Stilbum infection may be confined entirely to the woody portion of the fig tree.

Host Relations.—In the early part of April, 1933, a number of twigs of peach, plum, pear (Pineapple Sand variety), and seedling pecan trees were inoculated with *Stilbum cinnabarinum*. There was no evidence of infection on any of the trees except the pear. The fungus caused rather rapid blackening and dying of most of the inoculated young pear twigs of the current year's growth. Stilbum heads developed in the dead bark of some of the twigs several weeks after the twigs were killed, but they were much smaller, very pale pink, and not nearly so well developed as those produced on fig. There has been no indication of spread of the Stilbum from the young branches into the older portions of the pear trees, as it occurs in the fig.

A preliminary set of varietal inoculations was made in the spring of 1934. The following varieties were used: Celeste, Brunswick, Magnolia, Brown Turkey, and Green Ischa. There were 8 twigs on each of these varieties inoculated with each of 2 strains of *Stilbum cinnabarinum*. Appropriate controls were also used. After 45 days the twigs were examined. The Green Ischa, Brown Turkey, and Magnolia were severely injured, while infection was less severe in the Celeste and Brunswick. While these tests are only preliminary, they indicate that other fig varieties than the Celeste may be susceptible to Stilbum infection.

Since a very similar fungus has been found causing a canker of rose in Cuba, it was thought to be of some interest to make inoculations on this plant with the Louisiana Stilbum. A preliminary set of inoculations made on the climbing rose (Killarney variety) with two Stilbum cultures failed to produce any infection after 3 months, while fig branches inoculated at the same time with the same cultures were killed.

Control Measures.—Pruning out infected branches and painting the cut ends with ordinary outside paint has proved to be fairly effective in controlling Stilbum on fig trees that had only a few incipient infections. A group of 6 fig trees, 8 to 10 years old, on the property of Mr. Reuss (where Stilbum was first discovered in Louisiana) had a few infected twigs on each tree. These diseased twigs were cut out and a coating of heavy gray outside paint applied to the exposed surfaces. A year later, when the trees were examined, only 2 infected twigs were found on the entire group of 6 trees.

However, on an old, heavily infected tree the results were different. Several large diseased branches were removed from this tree and the cut ends were covered with a coating of heavy paint. This was done during the early part of April, 1933. Within 6 weeks the Stilbum heads were very numerous around the cut ends of 3 large branches and the fungus heads had grown directly through the heavy coating of paint in 2 cases. It is probable that these branches had not been cut back far enough to get beyond the infected wood.

While no definite control recommendations based on accurate data can be made at present, it is believed that some precautions can be taken which will reduce the damage caused by Stilbum on fig trees. If the trees are set out in the open, away from the shade of buildings, larger trees, etc., the chances for Stilbum infection are greatly reduced. Pruning out the interior of the trees so as to allow sunlight to penetrate as much as possible is also a good practice. If Stilbum-infected twigs are found, they should be removed, care being taken to cut back beyond the brown discolored bark. Painting the cut ends of the branches may also help to prevent outside infection.

SUMMARY

A new disease of fig, *Ficus carica,* caused by *Stilbum cinnabarinum* (Mont.) is described from Louisiana. Stilbum diseases are prevalent in the tropics, but this is apparently the first one described in the continental United States.

The Stilbum disease is widespread over southern Louisiana, and, under certain conditions, causes considerable damage to fig trees. Diseased branches usually have the characteristic small pink fungus heads, borne on short stalks growing on the bark.

The ascigerous stage, *Megalonectria pseudotrichia,* of *Stilbum cinnabarinum* has been definitely connected with the conidial or Stilbum stage.

Inoculation tests show that the fungus attacks fig twigs readily through slight wounds, but apparently not through the unbroken epidermis.

The Stilbum disease can be controlled in young trees by pruning out infected branches, but old, badly diseased trees should be destroyed.

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RELATION BETWEEN NUMBER, SIZE, AND LOCATION OF SMUT INFECTIONS TO REDUCTION IN YIELD OF CORN[1]

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INTRODUCTION

During recent years in many States severe losses from corn smut (*Ustilago zeae* (Beckm.) Ung.) have emphasized the economic importance of this disease. It is conservatively estimated that 2 per cent of the United States corn crop, or 55,000,000 bushels, is destroyed annually by smut (5). During the 3 years, 1931 to 1933, the losses from corn smut in Minnesota have ranged from 5 to 10 per cent (5).

The damage caused by corn smut is evident when seedlings or young plants are killed, ears are destroyed, or when the stalks are severely infected, but until recent years it has not been generally recognized that the presence of a smut gall on an otherwise apparently healthy plant may materially reduce its yield. Immer and Christensen (2, 3) and Jorgenson (4) found that the reduction in yield of inbred lines and F_1 crosses of corn due to a single smut infection was dependent upon the size and location of the smut gall on the plant. Garber and Hoover (1) found a higher percentage of barren stalks on smut-infected than on smut-free plants but were unable to demonstrate other significant decreases in yield resulting from smut infection.

Only meager information is available regarding the reduction in yield caused by two or more galls on the same plant or by ear infection. Furthermore, little is known regarding the effects of smut on normal varieties of corn.

The present paper summarizes the results on smut losses that have been obtained in Minnesota since the earlier publications by Immer and Christensen (2, 3). The tests were made particularly to determine the reduction in yield caused by two or more galls on the same stalk and by galls located on the ear. Studies on the effects of single smut galls, however, were continued in order to eliminate errors due to seasonal fluctuations and to check the accuracy of earlier investigations. In addition, tests were made on the indirect effect of smut infection on kernel luster and as a predisposing factor for ear rots.

MATERIALS AND METHODS

In 1932 and 1933 more than 1,800 comparisons were made between adjacent smutted and smut-free plants of dent corn consisting of 3-way

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crosses, double crosses, and top crosses; standard open-pollinated varieties and inbred lines were used also. All plants were grown in rows either with single plants spaced 1 foot apart or in hills of 2 to 3 plants. In the drilled corn each paired comparison consisted of a smut-infected and smut-free plant of the same variety, cross, or inbred line growing 1 foot apart; in the hill-planted corn the paired comparison consisted of 2 plants growing in the same hill. Since the hybrids and varieties used in this study were rather variable, only those paired plants that were similar in size were used. The ears from the smutted and smut-free plants were harvested and placed in paper bags, the ears from the smutted plants were marked, and the number, size, and location of the smut galls were recorded. The harvested ears were thoroughly dried at 170° F. and then weighed without shelling.

In 1932 no attempt was made to select pairs of plants with smut galls of specific size or location, but in 1933, in order to obtain a representative population for each gall of a particular size and location, only those pairs were harvested that were obtained with least frequency during the previous year. During both years approximately 600 comparisons were obtained between smut-free plants and plants infected with 2 or more galls; and over 1000 comparisons were obtained between smut-free and smut-infected plants having only a single gall. Statistical analysis of the data was made by Student's pairing method; each pair consisting of an adjacent smutted and smut-free plant.

EXPERIMENTAL RESULTS

The data obtained in this study are most conveniently classified into 3 parts: comparisons of smut-free plants and infected plants with a single smut gall; comparisons of smut-free plants with infected plants having multiple smut galls; and the relation of smut infection to kernel luster and to ear rots.

Single Smut Galls

The relation between yield and infection by small, medium, and large smut galls, at 9 specific locations on the plant, was studied with the varieties and crosses as shown in table 1. In the original analysis of the data, the results for the varieties and crosses were summarized separately for each of the 2 years, but since the varieties and crosses responded in a similar manner and no differences were observed between the 2 years, all of the data were combined to utilize a large number of comparisons.

From the data given in table 1 it is evident that the losses from smut infection are dependent upon the size and location of the smut gall. Small galls on the nodal shoots below the ear did not appreciably reduce the yield, but the losses from medium-size and large galls at the same locations were

TABLE 1.—*Relation between size and location of single smut galls on varieties and crosses to reduction in yield of ear corn*

Location of gall	Size[a]	No. of comparisons	Per cent reduction	Odds that loss is significant
Base	Small	23	2.4	2:1
	Medium	34	15.5	230:1
	Large	10	17.7	7:1
Shoot	Small	113	1.9	2:1
	Medium	119	18.2	250:1
	Large	35	50.2	>5,000:1
Sucker	Small	29	1.9	1:1
	Medium	15	3.3	2:1
	Large	4	26.0	3:1
Total below ear	Small	165	2.0	2:1
	Medium	168	16.5	152:1
	Large	49	41.7	>10,000:1
Main stalk	Small	28	16.3	1,750:1
	Medium	31	39.8	>10,000:1
	Large	25	99.0	>10,000:1
Neck	Small	126	11.2	300:1
	Medium	127	27.1	>10,000:1
	Large	31	75.9	>10,000:1
Tassel	Small	19	7.2	14:1
	Medium	9	88.4	>10,000:1
	Large	3	100.0	112:1
Total above ear	Small	173	11.6	249:1
	Medium	167	33.0	>10,000:1
	Large	59	86.7	>10,000:1
Ear tip	Small	46	−1.1	2:1
	Medium	40	19.4	>10,000:1
	Large	18	52.0	>10,000:1
Ear general	Small	18	10.7	6:1
	Medium	16	28.7	130:1
	Large	94	90.0	>10,000:1
Total ear infections	Small	64	2.2	2:1
	Medium	56	22.0	>10,000:1
	Large	112	83.4	>10,000:1
Leaf	Small	19	−6.2	4:1

[a] Small galls—up to 2 inches in diameter.
Medium galls—from 2 to 4 inches in diameter.
Large galls—over 4 inches in diameter.

highly significant. Small and medium-size galls on the suckers did not reduce yield, although large galls at this location reduced the yield by 26.0 per cent. The total of all single gall infections below the ear, namely, base, shoot, and sucker, caused a weighted average loss of 2.0 per cent for small, 16.5 per cent for medium, and 41.7 per cent for large galls.

Smut galls, particularly the medium and large-size galls, located above the ear were much more destructive than those below the ear, and frequently resulted in barren stalks. The difference in destructiveness of galls located above and below the ear is explained on the basis of the part of the plant

attacked. Infections below the ear were largely on ear buds, while infections above the ear were chiefly nodal or internodal and injured the main stalk.

Smut galls on the tassels[2] gave a surprisingly large reduction in yield. Since only 31 comparisons were obtained, it is not desirable to draw too definite conclusions as to the exact losses. Medium and large galls on the tassel were nearly always accompanied by barren stalks, but small galls caused no significant decrease in yield. Many of the small galls, however, consisted merely of a few scattered spikelet infections. These results are not in agreement with those obtained by Garber and Hoover (1), who found no reduction from tassel infection.

The statistical analysis in table 2 is made by comparing the average reductions in yield resulting from infection by small, medium, and large single galls above and below the ear. Differences of 3.7, 4.0, and 5.6 times the standard error, respectively, prove conclusively that smut galls above the ear caused the greater loss in yield. The data on the relative destructiveness of galls below and above the ear confirm the earlier results obtained by Immer and Christensen (2, 3) and Jorgenson (4).

There were two quite distinct types of ear infections. In one type, the smut gall was attached to the tip of the cob and in the other it was generally distributed over the ear and usually resulted from several kernel infections. These infection types have been designated in table 1 as "ear-tip" and "ear-general," respectively. They were compared separately with smut-free plants, and the results show that on the average the tip infections are less destructive than the general infections. The average yield of 46 plants with small galls on the ear tip was slightly more than that of the smut-free plants, but the difference in favor of the infected plants was not statistically

TABLE 2.—*Average reduction and average difference in yield in grams between paired smut-free and smut-infected plants*

Size of galls	Galls above the ear		Galls below the ear		
	Number of comparisons	Av. reduction in yield	Number of comparisons	Av. reduction in yield	Difference
Small	173	21.18 ± 2.99[a]	165	3.66 ± 3.69	17.52 ± 4.75
Medium	167	60.69 ± 5.14	168	31.81 ± 5.00	28.88 ± 7.17
Large	59	155.85 ± 7.66	49	77.08 ± 11.93	78.77 ± 14.17

[a] Standard errors.

[2] In case of tassel infection a so-called large gall consisted of a large number of closely aggregated spikelet infections; a small gall, of few scattered infections.

significant. Small galls of the general type on the ear gave a reduction of 10.7 per cent, although the difference was not statistically significant. Plants with medium and large galls of the ear-tip or ear-general type yielded significantly less than the smut-free plants used in making comparisons. Large galls, generally distributed, usually destroyed the entire ear, while similar sized galls on the ear tip reduced the yield 52.0 per cent. A statistical comparison between the average reduction in yield from small, medium, and large galls of the ear-tip and ear-general type gave differences that were not significant for small and medium galls but highly significant for large galls. The difference between the reduction in yield caused by general ear infection and ear-tip infection was 4.8 times the standard error.

Over 200 comparisons with single smut galls on inbred lines at different locations gave essentially the same relationship as shown with the varieties and crosses.

Multiple Smut Galls

Under normal field conditions plants with 2 or more smut galls occur quite frequently. At University Farm, in 1933, a study of the smut reaction of 40 varieties and crosses showed that 20–25 per cent of the infected plants had 2 or more smut galls. Since multiple galls may vary in position on an individual plant, the data were classified into 3 groups on the basis of smut locations, namely, those plants on which all galls were below the ear, those on which a part were above and a part below the ear, and those on which all galls were above the ear. Since the number of galls on each plant varied also, the data were classified into 2 groups on the basis of number of galls. This classification includes those plants bearing 2 galls and those bearing 3 or more galls per plant. According to size of galls per plant, the groups were divided into 5 classes, namely, small, small and medium on the same plant, medium (1 large and 1 small gall were classified as 2 medium galls), medium and large on the same plant, and large galls. The average percentage reduction from 600 paired comparisons of smut-free plants and plants with 2 or more galls is given in table 3.

Comparing plants with smut galls of similar size, the results show that infection below the ear gave the least reduction; plants in which a part of the galls were above and a part below the ear were next, and plants with all galls above the ear gave the greatest reduction in yield. Losses in yield from multiple infections are also dependent upon the size of the smut gall. Two small galls per plant below the ear, above the ear, and generally distributed over the plant reduced the yield 12.9, 28.9, and 13.9 per cent, respectively, while medium galls at these locations gave 3 times larger losses in yield. Large multiple galls usually caused complete stalk barrenness regardless of the position of the gall on the plant.

TABLE 3.—*Relation between size, number, and location of multiple smut galls on varieties and crosses to reduction in yield of ear corn*

Number and size of multiple galls[a]	Location of smut galls								
	All below the ear			Above and below the ear			All above the ear		
	Number of of comparisons	Per cent reduction	Odds that loss is significant	Number of of comparisons	Per cent reduction	Odds that loss is significant	Number of of comparisons	Per cent reduction	Odds that loss is significant
S, S	47	12.9	44:1	40	13.9	53:1	19	28.9	>10,000:1
S, S, S+	15	19.7	57:1	14	35.0	178:1	4	34.0	6:1
S, M	53	19.3	171:1	40	50.2	>10,000:1	24	37.5	> 7,000:1
S, S, M+ / S, M, M+	19	35.4	2,300:1	59	61.7	>10,000:1	6	67.6	59:1
M, M	67	44.4	>10,000:1	30	70.9	>10,000:1	40	83.5	>10,000:1
M, M, M+	31	79.6	>10,000:1	13	75.1	>10,000:1	4	86.5	43:1
M, L	22	78.9	> 7,000:1	9	78.6	>10,000:1	12	88.6	>10,000:1
M, M, L+ / M, L, L+	4	100.0	1,700:1	9	100.0	>10,000:1	.....	.....	.....
L, L	6	71.3	1,100:1	3	85.7	15:1	2	100.0	40:1
Total	274	39.2	>10,000:1	214	53.4	>10,000:1	111	62.5	>10,000:1

[a] S, S = 2 small galls; S, S, S+ = 3 or more small galls; S, M = 1 small and 1 medium gall, etc.

The relation between the number of galls and reduction in yield is shown by comparing losses from 2 galls with losses from 3 or more galls of similar size and location. Although the number of comparisons with 3 or more galls per plant is not large, it is evident that 3 galls caused greater reduction in yield than did 2 galls. On the average the increased reduction from 3 galls per plant is nearly one-third greater than for 2 galls, which indicates that losses from multiple infections are nearly directly proportional to the number of galls per plant. This relationship holds for small and medium galls only, since losses from 2 large galls usually approach 100 per cent.

A comparison of the data in table 1 with those in table 3 clearly shows that multiple galls reduce yield more than do single galls. Single small galls below the ear, chiefly on shoots and suckers (Table 1), reduced the yield 2.0 per cent, while 2 small galls and 3 or more galls below the ear resulted in losses of 12.9 and 19.7 per cent, respectively. The difference in loss between 1 small gall and 2 small ones is 2.1 times the standard error. The same general relationship is shown between single, medium, and large galls and multiple, medium, and large galls; the difference in reduction of yield between 1 and 2 medium galls below the ear is 5.0 times the standard error and between 1 and 2 large galls below the ear 2.7 times the standard error. Similar comparisons between the reduction in yield from 1 and 2 galls above the ear (chiefly main stalk and neck) show that multiple galls were significantly more destructive. A general summary comparing losses from single and multiple galls is given in table 4.

In addition to the losses from single and multiple galls, it was found that among the varieties and crosses grown at University Farm in 1931, 1932, and 1933, .7 per cent of the infected plants was killed as a result of smut infection.

Seventy comparisons with multiple infections were obtained among the inbred lines. In general, the results agreed with those shown for the varieties and crosses.

Ear Characters Associated With Smut

In 1932 and 1933 notes on ear luster were taken, and in 1933 data also were obtained on ear rots between paired comparisons of smut-infected and smut-free plants. No attempt was made to assign a numerical value for either ear luster or ear rots. The better luster and the least ear rot were determined for each paired comparison without knowing which ear was produced by the smut infected plant. If there was no relationship between smut infection and either luster or ear rots, smutted plants should have the best luster in 50 per cent of the cases and the least ear disease in 50 per cent

TABLE 4.—*General summary of reductions in yield from single and multiple smut galls*

Location of smut gall	Size of gall and percentage reduction									
	n.[a]	Small	n.	Small and medium	n.	Medium	n.	Medium and large	n.	Large
Single infections										
Below the ear	165	2	.....	.....	168	17	.....	.....	49	42
Above the ear	173	12	.....	.....	167	33	.....	.....	59	87
Ear	64	2	.....	.....	56	22	.....	.....	112	83
Multiple infections										
All below the ear	62	14	72	24	98	56	26	82	7	85
Above and below the ear	54	23	99	57	43	74	18	89	3	86
All above the ear	23	30	30	44	44	84	.....	.....	2	100

[a] Number of paired comparisons.

of the comparisons. On this basis, statistical treatment of the data could then be made by means of the X^2 test for a 1:1 ratio.

The data given in table 5 are based on those comparisons where the smut-infected and smut-free plant both produced an ear, and includes only those comparisons where distinct differences were shown in ear luster and in ear rots.

TABLE 5.—*Relation of smut infection to ear luster and to infection with ear-rotting organisms*

Location of smut gall	Ear luster			Ear rots		
	No. of comparisons	Percentage of ears with best luster		No. of comparisons	Percentage of ears with most disease	
		Smut-infected	Smut-free		Smut-infected	Smut-free
Single infections						
Below the ear	244	38	62	.....	.....	.....
Above the ear	161	36	64	79	66	34
Ear smut	73	28	72	52	90	10
Multiple infections	218	29	71	106	80	20
Total	701	34	66	237	78	22

The results of these comparisons clearly show that ears borne on smutted plants tend to have a poorer luster than those from their smut-free checks. In 244 comparisons involving single smut galls below the ear, the ear from the smutted plant had the better luster in 38 per cent and the poorer luster in 62 per cent of the cases. The X^2 test based on numbers gave odds greater than 100:1 that the observed frequency deviated from a calculated 1:1 ratio. In all other simple smut infections and in multiple infections the odds were nearly 100:1 to greater than 100:1 that the ear borne on the smut-free plants had the better luster.

The association between ear rots and smut infections in 1933 was even more striking than the relation between smut infection and luster. In 52 comparisons with ear-smutted plants 90 per cent of the smutted ears also had the most ear rots. In the cases involving smut galls below the ear, above the ear, or in multiple infections, the odds based on the X^2 test were over 100:1 that the ear from the smut-free plant had the least ear rot. These results indicate that the plants were apparently predisposed to ear rots following smut infection. Smut infection on the ear apparently affords an accessible avenue for the entrance of ear-rotting organisms, and this may partly account for the relatively large amount of ear rots on smutted ears.

DISCUSSION

The results of these studies may be of considerable importance in determining the losses due to smut. In making a general field survey of losses from corn smut it probably would not be desirable to record the number, size, and position of the smut gall as specifically as is given in the present paper. However, these studies indicate that the average reduction in yield caused by smut infection, irrespective of size, or location of smut galls, was approximately 25 per cent for single galls and about 50 per cent for multiple galls. On the basis of the percentage of plants with multiple and single galls, the average reduction in yield per plant due to smut is estimated to be approximately 30 per cent. Losses also result indirectly from smut infections by lowering the quality of the corn and by increasing the likelihood of mold attack on the ears.

The results of these investigations emphasize the need of breeding for smut resistance in corn. Highly smut-resistant inbred lines of corn have been obtained fairly easily by inbreeding and selection at University Farm. The recombination of these smut resistant lines into desirable hybrid combinations offers the most feasible method of reducing losses from corn smut.

SUMMARY

In 1932 and 1933, at University Farm, St. Paul, Minnesota, over 1800 paired comparisons were made between smut-free and smut-infected corn plants, of which there were hybrids, varieties, and inbred lines. It was shown that the extent of loss in yield of ear corn resulting from smut infection was dependent upon the number, size, and location of the smut galls.

The reduction in yield from multiple galls was significantly greater than that from a single smut gall of similar size and location. On the average, single smut galls reduced the yield about 25 per cent, and multiple galls approximately 50 per cent.

Single or multiple smut galls on the main stalk and neck above the ear were about twice as destructive as smut galls of similar size and number located below the ear, usually on shoots or suckers. Medium-size single or multiple smut galls above the ear frequently caused barren stalks, while large galls above the ear usually resulted in barren stalks.

In 1932 and 1933 approximately .7 per cent of smut-infected plants in all the varieties and crosses grown at University Farm were killed prematurely by smut.

Smut galls on the tip end of the ear were less destructive (loss 23 per cent) than galls of similar size more generally distributed through the ear (loss 43 per cent). Large ear galls usually resulted in barren stalks but small galls on the ear did not reduce yield significantly.

Medium and large smut galls on the tassels caused heavy reduction in yield. Small smut galls on the tassel were not injurious.

Ears from smut-infected plants tended to have a poorer kernel luster than those from smut-free plants, and also were more likely to be infected with ear rots.

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DUSTING AND SPRAYING EXPERIMENTS FOR THE CONTROL OF SUGAR-BEET LEAF SPOT IN SOUTHERN MINNESOTA[1]

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Leaf spot (*Cercospora beticola* Sacc.) of sugar beets occasionally is quite destructive in some areas in southern Minnesota. Hitherto, few experimental data have been obtained regarding direct control of this disease in nonirrigated sections, although it has been effectively controlled by Coons, Stewart, and Larmer[3] and Townsend[4] under conditions of irrigation. The following experiments were made in 1931 and 1932 to determine the relative efficiency of Bordeaux mixture and copper sulphate-lime dust in controlling the disease.

The experiments were made in commercial beet fields near Chaska, Minnesota. Bordeaux mixture (4–4–50) was used for spraying and monohydrated copper sulphate and lime (20–80) for dusting. The Bordeaux mixture was always freshly made from stock solutions. Hydrated lime, such as is sold commercially for finishing plaster, was used for both the liquid and dust fungicides. The liquid applications were made with a 3-gal. compressed-air hand sprayer, equipped with a 3-ft. extension rod and angle nozzle. The dust was applied with a "Hudson" hand duster. Care was taken to insure thorough coverage of all plants at each application. The first applications were made when leaf spot first appeared in the fields (about July 15) and the later applications were made at approximately 2-week intervals.

Plots, 8 rows wide and 100 feet long, were used for all experiments. Each treatment was replicated 5 times. In 1931 the sprayed plots in Field A were arranged in the form of a Latin square; all others were arranged as

[1] The data presented in this paper were obtained in cooperative investigations by the Division of Sugar Plant Investigations, Bureau of Plant Industry, United States Department of Agriculture, and the Division of Plant Pathology and Botany of the Minnesota Agricultural Experiment Station. Paper No. 1240 of the Journal Series of the Minnesota Agricultural Experiment Station.

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[3] Coons, G. H., Dewey Stewart, and F. G. Larmer. The sugar-beet leaf-spot disease and its control by direct measures. U. S. Dept. Agr. Circ. 115. 1930.

[4] Townsend, C. O. Leaf-spot, a disease of the sugar beet. U. S. Dept. Agr. Farmers' Bull. 618. 1922.

randomized blocks. In 1932 the Latin-square arrangement of plots was used for all tests. Four rows were left between the dusted plots to minimize as far as possible the effect of any drifting dust. The 6 inside rows of each plot were harvested. Beets adjacent to obvious skips in the row were discarded and an attempt was made to take beets with normal competition on all sides in determining the yield. All yields are computed on the basis of a 100 per cent stand of beets in the plots. Three 20-beet samples were taken from each plot for determination of percentage sucrose and apparent coefficient of purity. For the former the Sachs-Le Docte method was followed, while the apparent coefficient of purity was determined from the refractometer value and the sucrose percentage of a sample of pulp.

All data were analyzed by the analysis-of-variance method, as outlined by Fisher,[5] the value of z at the 5 per cent point being taken as the criterion for significance of the experiments.

To determine which mean differences were statistically significant, the various means for treatment were compared with those for nontreatment and with each other. In these comparisons the standard errors of the difference between 2 means were determined.[6] To determine the value necessary for significance the appropriate value for t, as given in Fisher's tables, was found and the standard error of the difference multiplied by this figure. This value of t takes into consideration the number of degrees of freedom for error. Individual analyses of variance were made of each test.

Experiments in 1931

The experiments in 1931 were made in fields located near the banks of the Minnesota River, owned by William Schalow and Fred Rauschfleisch. For convenience the fields are designated A and B, respectively. Both fields had a fairly uniform stand and had previously grown sugar beets under short rotations.

In field A leaf spot was scarce on July 16 when the first applications were made, with only 1 to 6 spots per leaf. Two weeks later (July 29), in the untreated portion of the field, the number of spots had increased 4 or 5 times per leaf, and distinct infection foci were evident. By August 13, these foci were more numerous, and the disease spread from these centers to a distance of approximately 4 feet. The prevalence of leaf spot increased rapidly and, by August 27, the condition could be called epidemic. On this date many lower leaves were dead. During the ensuing 2-week period new leaves were produced, and they were in turn destroyed by a second out-

[5] Fisher, R. A. Statistical methods for research workers. Ed. 4, rev. and enl., 307 pp. illus. Oliver and Boyd, Edinburgh and London. 1932.

[6] The standard error of the difference between two means = $\sqrt{\frac{\sigma^2 \times 2}{n}}$.

break of the disease, so that by September 10 only the center whorls of small leaves on the majority of the plants were green.

In field B the disease had not become epidemic by harvest. Although most of the plants in the non-treated plots were infected, there was very little leaf injury.

The results of dusting and spraying in 1931 are summarized in table 1.

Dusting Tests

Analyses of the yields obtained from the dusting tests show no significant differences due to treatment. In field A the disease situation was such that significant differences were to be expected because the disease was controlled. But because of unfortunate circumstances, doubtless related to the stand condition in at least 3 plots, the yields computed from the plots departed widely from the average of the replications. It is to be assumed that, because of poor stand, beets not wholly subjected to normal competition were harvested and that the average weight determined per individual root was too high, thus giving an extremely high tonnage value when this average root weight was multiplied by the number of roots per acre in a perfect stand. When the 2 replications in which these questioned values occur were discarded and the data computed on the basis of the remaining 3 blocks, the results were significant. From these average yields, progressive increases in tonnage with the greater number of applications of dust are obtained. Therefore, under these conditions, it seems fair to state that, although the test as a whole was inconclusive, there are definite trends indicating control of leaf spot and gains in yield. The data on sucrose percentage and apparent coefficient of purity do not indicate significant increases.

In field B the disease caused only slight injury, and significant differences in yield were not obtained. Similarly, the data for sucrose percentages and for apparent coefficients of purity were not significant.

Spraying Tests

Analysis of the yields obtained from the spraying test in field A demonstrates that the variance assignable to treatments was statistically significant for the experiment as a whole. The standard error of the difference of any 2 means in this test was found to be 0.87 tons, and this figure, when multiplied by the appropriate t value (2.179), equals 1.89 tons. This figure is taken as the tonnage difference between any 2 means required to reach the 5 per cent level of significance. By applying this test to the data the average yield, where 2 applications of Bordeaux mixture were made, is not significantly different from that of the control (nonsprayed), but the averages for 3, 4, and 5 applications are significantly higher than from the

TABLE 1.—*Results of dusting and spraying tests with sugar beets in Field A at Chaska, Minnesota, in 1931. The disease was in epidemic amount by August 27*

Kind of treatment	Number of applications	Yield or roots[a]		Sucrose		Apparent coefficient of purity	
		Computed (per acre)	Increase over control	Determined value	Increase or decrease over control	Determined value	Increase or decrease over control
		tons	*tons*	*per cent*	*per cent*	*per cent*	*per cent*
Dust	0	17.89	.....	13.43	.....	86.12	.....
	3	21.07	+3.18	13.71	+0.28	86.10	−0.02
	4	21.07	+3.18	12.91	−0.52	85.88	−0.24
	5	24.30	+6.41	13.04	−0.39	87.58	+1.46
Difference required for significance	.....	.....	[b]		[d]		[d]
Spray	0	19.69	.....	13.94	.....	86.86	.....
	2	20.07	+0.38	14.95	+1.01	87.31	+0.45
	3	21.74	+2.05	14.61	+0.67	88.02	+1.16
	4	22.23	+2.54	14.75	+0.81	88.31	+1.45
	5	22.61	+2.92	14.42	+0.48	88.30	+1.44
Difference required for significance	.....	.....	1.89		[b]		[c]

[a] Yields computed on basis of 100 per cent stand from plot data.

[b] Variance due to error was greater than that due to treatment; therefore, the data are not significant.

[c] Value of observed z did not reach the value of the calculated z at the 5 per cent point.

[d] Data not analyzed, since data for yields were not significant.

control. Three applications were no better than 2, although the difference was suggestively large. Further, 4 and 5 applications proved to be superior to 2 applications, but were not significantly different among themselves. From this experiment, made in fields in which leaf spot was epidemic, it can be concluded that 3, 4, and 5 applications of Bordeaux mixture controlled leaf spot and resulted in significant tonnage increases as compared with the nonsprayed plots. The analyses of sucrose percentages and apparent coefficients of purity demonstrated that they were not significant; therefore, significant differences are not to be expected from the test.

Since leaf spot did not become very prevalent at any time during the growing season in field B, the average yields, sucrose percentages, and apparent coefficients of purity for the various spraying treatments were not significant.

The data obtained in 1931 in the field where leaf spot was epidemic failed, as previously mentioned, to demonstrate that dusting effectively controlled the disease. The results, however, are interpreted as

indicative of a trend in favor of the dusted plots, and they show that favorable results might have been obtained had the stand in the plots been more uniform at the outset. The results with Bordeaux mixture were conclusive in that statistically significant differences were obtained where 3 or more spray applications were made. Because of these indications, experiments of the same type were made in the following season.

EXPERIMENTS IN 1932

In 1932 the dusting and spraying tests were made in fields owned by Arthur Le Mieux and William Ess, both fields consisting of river-bottom land near Chaska, Minnesota. These fields have been designated as A and B, respectively. The stands in both were exceptionally good.

Leaf spot was exceedingly destructive in both fields, causing burning of all lower leaves in the nontreated plots. The plants in the sprayed plots in both fields were well protected from infection and in marked contrast to the nonsprayed plants. The protective value of Bordeaux was evident on visual field inspection, the nonsprayed plants being severely burned, while sprayed plants were green and relatively disease-free. The dusted plants were protected to a certain extent, but apparently not so fully as the sprayed ones. The results are summarized in table 2.

Dusting Tests

Analysis of the yields from the dusted plots in field A show that 2, 4, and 5 applications did not bring about yields significantly higher than those obtained in the nondusted plots. The plots receiving 3 applications appear to yield significantly more than those receiving no dust or 2 applications. This probably is due to chance rather than to superiority of this particular treatment, because the plots receiving 1 and 2 additional applications do not show similar superiority over the control. It should be remarked that the gains in yield over the control, in the case of the plots receiving 4 and 5 applications, are suggestively large and indicate that the dusting was of considerable value. There was a progressive increase in sucrose percentage with an increase in the number of applications of dust. The dusted plots, irrespective of number of applications, were superior in percentage of sucrose to the nondusted plots. Those receiving 4 and 5 applications did not differ significantly in percentage of sucrose, but apparently were significantly better than those with 2 applications. Apparent coefficients of purity were found to be not significantly different; therefore, only gross sugar per acre was calculated. There was increased gross sugar per acre from all dusted plots as compared with the nondusted ones. The gross sugars of plots receiving 3, 4, and 5 applications did not differ statistically among

TABLE 2.—*Results of dusting and spraying tests with sugar beets at Chaska, Minnesota, in 1932*

Kind of treatment	Number of applications	Yield of roots[a]		Sucrose		Apparent coefficient purity		Estimated gross per acre	
		Computed, per acre	Increase over control	Determined value	Increase over control	Determined value	Increase over control	Computed[d] per acre	Increase over control
				Field A: Disease in epidemic amount					
		tons	*tons*	*per cent*	*per cent*	*per cent*	*per cent*	*lbs.*	*lbs.*
Dust	0	16.75	.....	15.54	.....	86.34	.....	5211.2	.....
	2	16.86	0.11	16.20	0.66	88.10	1.76	5472.0	260.8
	3	18.59	1.84	16.42	0.88	87.20	0.86	6112.2	901.0
Difference required for	4	17.78	1.03	16.74	1.20	87.32	0.98	5953.2	742.0
	5	17.82	1.07	17.22	1.68	87.28	0.94	6133.8	922.6
significance	.....	.....	1.17		0.57		[c]		556.8
Spray	0	17.16	.....	15.30	.....	86.42	.....	5248.6	.....
	2	17.21	0.05	16.02	0.72	86.68	0.26	5514.2	265.6
	3	17.84	0.68	16.24	0.94	87.46	1.04	5797.0	548.4
Difference required for	4	18.12	0.96	16.40	1.10	86.44	0.02	5945.2	696.6
	5	18.57	1.41	16.10	0.80	86.68	0.26	5984.2	735.6
significance	.....	.....	0.84		0.33		[c]		265.8
				Field B: Disease in epidemic amount					
Dust	0	27.16	.....	14.58	.....	83.62	.....	7910.4	.....
	2	31.22	4.06	14.94	0.36	83.66	0.04	9329.0	1418.6
	3	32.69	5.53	15.40	0.82	86.16	2.54	10061.8	2151.4
Difference required for	4	31.34	4.18	15.68	1.10	84.94	1.32	9232.0	1321.6
	5	31.40	4.24	15.90	1.32	84.44	0.82	9956.0	2045.6
significance	.....	.....	3.50		0.32		[b]		1394.8
Spray	0	20.39	.....	14.74	.....	82.85	.....	6010.2	.....
	2	21.75	1.36	15.30	0.56	83.52	0.67	6654.8	644.6
	3	22.37	1.98	16.02	1.28	83.74	0.89	7175.2	1165.0
Difference required for	4	23.34	2.95	16.12	1.38	84.16	1.31	7519.8	1509.6
	5	23.81	3.42	16.20	1.46	83.72	0.87	7714.0	1703.8
significance	.....	.....	1.16		0.61		0.71		447.9

[a] Yields computed on basis of 100 per cent stand from plot data.
[b] Variance due to error was greater than that due to treatments; therefore, data are not significant.
[c] Value of observed z did not reach the value of the calculated z at the 5 per cent point.
[d] The estimated gross sugar per acre was obtained by multiplying individual plot yields in pounds per acre by mean percentage sucrose and dividing by 100.

themselves but were significantly greater than those of the plots with only 2 applications.

It is concluded from this test that yield was probably favorably influenced by 3 or more applications of dust and the percentage of sucrose definitely so. The cumulative effect of the favorable influence on these 2 factors is shown by the results obtained for gross sugar per acre, in which 3 or more dust applications are seen to bring about significant increases.

The analysis of the yields of roots from the dusted plots in field B showed that the experiment was significant and that all dust applications were significantly better than the control, but probably about equally effective. The average percentages of sucrose from the dusted plots were significantly higher than from the control and appeared to increase with the number of applications. No significant differences between the apparent coefficients of purity could be demonstrated. The analysis of the data for gross sugar per acre indicates significant increases over the control from 2, 3, 4, and 5 applications, the greatest increase being obtained in the plots receiving 5 applications of copper sulphate-lime dust.

Spring Tests

In field A yields from plots sprayed 2 or 3 times are not significantly higher than from the control, whereas 4 and 5 applications of Bordeaux mixture are about equally effective and are superior to 2 or 3 applications. It appears that the average percentage of sucrose of the sprayed plots, regardless of number of applications, were significantly greater than the average of the nonsprayed plots. The gains over the control increase progressively as the number of applications are increased. The extent of these gains closely approaches the amount required for significance and seems to indicate increased control of leaf spot. The value of z for analysis of the apparent coefficients of purity was below the 5 per cent point, and it is concluded that significant effects attributed to treatment were not obtained. The averages for gross sugar per acre indicate that the increases resulting from 2, 3, 4, and 5 applications were significant when compared with the nonsprayed plots. Four and 5 applications result in significant increases when compared with 2 applications, and 5 applications did not give an average value significantly larger than 4 applications.

The observed value of z for the analysis of the yields from the spraying test in field B greatly exceeded the calculated value of z at the 1 per cent point, so it is concluded that the results are highly significant. It appears that the greatest increase in tonnage resulted from 5 applications of Bordeaux mixture, but the yields of 2, 3, and 4 applications are all significantly greater than those of the nonsprayed plots. The analysis of the percentages of sucrose demonstrates that significant differences exist. All the

TABLE 3.—*Mean yields, percentages of sucrose, apparent coefficients of purity, and estimated gross sugar per acre from 2 dusting tests and 2 spraying tests at Chaska, Minnesota, in 1932*

Kind of treatment	Number of applications	Yield of roots		Sucrose		Apparent coefficient of purity		Estimated gross sugar per acre	
		Mean	Increase over control	Mean	Increase over control	Mean	Increase over control	Mean	Increase over control
		tons	*tons*	*per cent*	*per cent*	*per cent*	*per cent*	*lbs.*	*lbs.*
Dust	0	21.95	.....	15.06	.....	84.98	.....	6560.8	.....
	2	24.04	2.09	15.57	0.51	85.88	0.90	7400.5	839.7
	3	25.64	3.69	15.91	0.85	86.68	1.70	8087.0	1526.2
	4	24.56	2.61	16.21	1.15	86.13	1.15	7592.6	1031.8
	5	24.61	2.66	16.56	1.50	85.86	0.88	8044.9	1484.1
Difference required for significance	.....	.....	1.73		0.31		1.52		704.5
Spray	0	18.77	.....	15.02	.....	84.63	.....	5629.4	.....
	2	19.48	0.71	15.66	0.64	85.10	0.47	6084.5	455.1
	3	20.15	1.38	16.13	1.11	85.60	0.97	6486.1	856.7
	4	20.73	1.96	16.26	1.24	85.30	0.67	6737.5	1108.1
	5	21.19	2.42	16.15	1.13	85.20	0.57	6849.1	1219.7
Difference required for significance	.....	.....	0.67		0.32		0.66		244.3

sprayed plots, except possibly those receiving 2 applications, are significantly better than the nonsprayed plots. Furthermore, 3, 4, and 5 applications are statistically superior to 2, but these applications do not differ among themselves. All sprayed plots except those receiving 2 applications are significantly better than the nonsprayed plots when apparent coefficients of purity are compared, but, apparently, they do not differ significantly among themselves. A consideration of gross sugar per acre indicates that the sprayed plots are significantly better than the nonsprayed ones. Three, 4, and 5 applications resulted in significant increases when compared with 2 applications. Three and 4 applications were found not to differ significantly, but 5 applications are apparently better than 3. The results of spraying in this field, as a whole, indicate that 4 or 5 applications result in outstandingly higher increases.

Summary of the 1932 Tests

Since the experiments in both fields in 1932 were arranged in a like manner and leaf spot was epidemic in both fields, the data have been averaged and are presented in table 3.

The mean yields from the 2 dusting tests in both fields demonstrate that 2, 3, 4, or 5 applications of copper-lime dust are significantly superior to no treatment. The percentages of sucrose for all applications are significantly higher than the nontreated plots. There is a progressive increase in percentage of sucrose with increase in number of applications. A comparison of the apparent coefficients of purity show that an increase, when compared with the controls, was obtained with only 3 applications of dust. It is thought that this probably is due to chance, since plots receiving 4 and 5 applications are not significantly superior to the nondusted ones. The yield of gross sugar per acre was increased significantly over the controls by all applications.

The results of mean yields from the 2 spraying tests show that all applications give significantly greater yields than those from the nonsprayed plots. Three applications are significantly better than 2, but not superior to 4. Five applications of spray give significantly higher yields than either 2 or 3, but are not superior to 4, although the difference closely approaches significance. Percentages of sucrose for all applications of spray are significantly better than the control and 3, 4, and 5 applications are distinctly superior to 2, but they are not significantly different one from the other. Comparison of mean differences of the apparent coefficients of purity shows that no significant increase results from 2 applications of Bordeaux mixture, whereas 3 and 4 applications are both superior to the control; 5 applications closely approach significance. The values of the apparent coefficients of purity of these 3 applications, however, are not significantly dif-

ferent one from another. Progressively significant increases in the yield of gross sugar per acre were obtained with 2 and 3 applications when compared with the control. Four and 5 applications are not significantly different, but are decidedly better than the control and 2 or 3 applications.

From the data in table 3, the percentage increase in yield for 5 applications of dust over the control is 12.12 per cent, whereas for 5 applications of spray it is 12.89 per cent. Thus it appears that the 2 treatments probably were equally effective in controlling leaf spot.

SUMMARY

This report presents the results of 2 season's experiments on the efficiency of copper sulphate-lime dust (20–80) and Bordeaux mixture (4–4–50) for the control of Cercospora leaf spot in the vicinity of Chaska, Minnesota.

In 3 tests, made under epidemic conditions of the disease, significant increased yields when compared with the controls, were obtained in all spraying tests, whereas significant increases from dusted plots were obtained in only 2 tests; the third test, however, approached significance.

From field observation, the dusted plots had a somewhat lower measure of leaf-spot control than the sprayed plots, but a comparison of only statistically significant data from dusting and spraying tests indicates that the percentage increase in yield due to dusting and spraying is nearly identical.

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ANTAGONISTIC ACTION OF TRICHODERMA ON RHIZOCTONIA AND OTHER SOIL FUNGI[1]

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It has been demonstrated by a number of workers, that antagonistic relationships exist between many microörganisms, and in a few cases the phenomenon has been observed in plant pathogens. Sanford and Broadfoot (5) isolated 6 species of fungi and 15 species of bacteria that were able markedly to suppress the pathogenicity of *Ophiobolus graminis,* and Vasudeva (6) found that *Botrytis allii* inhibited decay in apples caused by *Monilia fructigena.* Weindling (7) recently reported that *Trichoderma lignorum* is parasitic on Rhizoctonia and several other fungi and suggests the possibility that this fungus might be used to control certain soil-borne pathogens. Brown (2) has found also that the presence of Trichoderma may cause a reduction in the amount of Texas root rot induced by *Phymatotrichum omnivorum.*

During the course of a series of greenhouse and laboratory studies on seed decay and seedling damping off caused by Rhizoctonia and Pythium, a number of tests were conducted that corroborate the observations made by Weindling concerning the antagonism between Trichoderma and Rhizoctonia and that have thrown some light on the probable nature of the antagonism in this case. The results of these greenhouse and laboratory tests are briefly presented.

GREENHOUSE STUDIES

In the first test an unsterilized potting soil was placed into half-gallon glazed earthenware pots, 24 of which were inoculated heavily with a pure culture of Rhizoctonia and 24 with Pythium. Twelve pots from each of these series were given an additional heavy inoculation with a pure culture of *Trichoderma lignorum* grown on sterilized whole oats.

Three days after inoculation half of the pots were planted with cucumber and half with garden pea seeds and allowed to remain in a warm, moist greenhouse where conditions were favorable for seed decay and damping off. In this test, the results of which are presented in the first part of table 1, it will be seen that, in the case of cucumber, the amount of seed decay and damping off was appreciably reduced in the series inoculated with Trichoderma, thus indicating that the presence of this fungus had in some way interfered with the parasitic action of the Pythium and Rhizoctonia. In

[1] Journal Series paper of the New Jersey Agricultural Experiment Station, Department of Plant Pathology.

the pots planted to peas the results were less marked, the Rhizoctonia series again showing beneficial effects from the Trichoderma, while the Pythium series gave negative results.

After removing the seedlings in the above test the pots were replanted to the same crop without further soil treatment in order to determine if the effect of the Trichoderma would persist for any length of time. At the

TABLE 1.—*Percentage of normal seedlings obtained from seeds planted in soil inoculated with Pythium or Rhizoctonia used alone or in combination with Trichoderma. Counts 14 days after planting.*

Seed	Inoculum			
	Pythium and Trichoderma	Pythium alone	Rhizoctonia and Trichoderma	Rhizoctonia alone
		First planting		
Cucumbers (90 seeds)	36.7	7.8	33.3	17.8
Peas (60 seeds)	34.0	40.0	53.8	41.7
		Second planting		
Cucumbers (90 seeds)	72.0	49.9	81.1	37.8
Peas (60 seeds)	55.0	30.0	55.0	31.7

time the second planting was made a month had elapsed since the inoculations. The results from this test, as shown in the second part of table 1, again showed a marked reduction of seed decay and damping off of both cucumbers and peas in the soils containing Trichoderma.

Another and similar test was conducted with soil previously sterilized with formaldehyde. After thorough aeration, the soil was placed into 72 pots arranged into 6 series of 12 pots each and inoculated as follows: Series 1—Pythium alone; Series 2—Rhizoctonia alone; Series 3—Trichoderma alone; Series 4—Pythium and Trichoderma combined; Series 5—Rhizoctonia and Trichoderma combined; and Series 6—Sterilized oats alone. The fungi in each case were grown on sterilized whole oats and both fungus and substratum were used as the inoculum.

Three days after inoculation the pots were planted to cucumbers or peas, 6 pots for each treatment, as in the former tests. The results, presented in table 2, again show a marked increase in the number of healthy seedings obtained where Trichoderma was used with Pythium or Rhizoctonia as compared with the series where the pathogens were used alone. As in the first tests, cucumbers again showed the greatest benefits from the presence of the Trichoderma.

The results of these greenhouse tests coincide with those obtained by Weindling (7) relative to the antagonistic action of Trichoderma on Rhizoc-

tonia and Pythium. They show clearly that the Trichoderma interferes with the normal activities of these pathogens, but they give no suggestions as to how this antagonism is brought about.

TABLE 2.—*Per cent of normal seedlings obtained from seed planted in Formalin-sterilized soil, subsequently inoculated with Pythium and Rhizoctonia, used alone or in combination with Trichoderma. Counts 14 days after planting*

Seed	Inoculum					
	Sterile oats	Trichoderma alone	Pythium and Trichoderma	Pythium alone	Rhizoctonia and Trichoderma	Rhizoctonia alone
Cucumber (90 seeds).....	80.0	80.0	87.8	31.1	87.8	33.3
Peas (60 seeds).....	46.7	40.0	53.3	23.3	61.7	36.7

PHYSIOLOGICAL STUDIES

After it had been found that Trichoderma seems to inhibit the parasitic activities of Rhizoctonia and Pythium in pot tests under greenhouse conditions, a number of laboratory experiments were conducted to further study the nature of this antagonistic action.

Porter (4), Broadfoot (1), Sanford and Broadfoot (5) and Fawcett (3), working on the association of certain organisms, have suggested that the inhibitions produced are caused by some toxic or enzymatic substance elaborated during the growth of the organism; and Weindling (7) states that *Trichoderma lignorum* parasitizes *Rhizoctonia solani* by means of some diffusible toxic or enzymatic substance. Very few statements are made by these workers, however, that give any indication as to the stability or other physiological properties of the toxic products formed. The following laboratory experiments, conducted with *Trichoderma lignorum*[2] and a strain of *Rhizoctonia solani* isolated from Lima beans, give some information along this line.

Preliminary Tests.—In a preliminary study, dextrose-agar plates were inoculated on opposite sides with Trichoderma and Rhizoctonia to observe whether an antagonistic zone would be produced between the 2 colonies. As the growth spread in these plates, it was noticed that the Rhizoctonia stopped growing when the Trichoderma mycelium approached within a few millimeters; the Trichoderma, on the contrary, spread over the Rhizoctonia mycelium as though the latter were not present. Such antagonism between

[2] This fungus has the general characteristics of *T. lignorum*, but the species has not been identified with certainty.

fungi grown in adjacent colonies in solid media has been observed by many and is of interest here only in that it demonstrates that the Trichoderma used in these experiments is antagonistic to the Rhizoctonia when grown in artificial media. Another preliminary experiment was conducted with flasks containing 100 cc. of dextrose broth.[3] These were inoculated with Trichoderma and Rhizoctonia alone or with the 2 organisms together to see which organism would dominate the other. After 4 days' incubation, the pure Rhizoctonia and the pure Trichoderma cultures showed excellent growth. Where Rhizoctonia and Trichoderma were together in the same culture, it was clearly evident that the latter had completely dominated. This was shown by lack of browning of the mycelium and of the nutrient solution, which was characteristic of all cultures containing Rhizoctonia alone. The mixed cultures appeared to be similar to the pure cultures of Trichoderma in every respect. Both of these preliminary experiments show that there is some inhibiting action of the Trichoderma on the growth of Rhizoctonia.

Toxicity of Filtrate from Trichoderma Culture. Several experiments were conducted to determine whether the filtrate from a liquid Trichoderma culture would inhibit the growth of Rhizoctonia or whether the inhibition occurs only in close proximity to the living Trichoderma cells. The 2 organisms were grown separately in dextrose broth for a period of 5 days, after which the luxuriant fungous mats were filtered off and the filtrate sterilized by passage through a Seizts filter. These filtrates were transferred to sterile flasks and half of each series inoculated with Rhizoctonia and the other half with Trichoderma. Control flasks containing fresh nutrient media also were inoculated with each organism. The results were very significant. In every case where the Rhizoctonia was inoculated into the filtrate from the Trichoderma cultures, there was no growth, (Fig. 1, 1). In all other cases the organisms grew as well as in the control flasks, showing definitely that there was no lack of nutrients in the filtrates, (Fig. 1, 2). It was also evident that Rhizoctonia produced no toxic product that was injurious to either Rhizoctonia or Trichoderma. (Fig. 1, 3, 4.) This same experiment was conducted 3 times, always with the same results. This is conclusive evidence that an active principle inhibitory to the growth of Rhizoctonia is elaborated into the medium by the Trichoderma and that the immediate presence of the organism is not necessary to cause the inhibition.

Destruction of Toxic Principle by Heat.—A test was conducted to determine whether the active principle produced by the Trichoderma is destroyed by heat sterilization. Filtrates from Trichoderma and Rhizoctonia cultures were prepared as before. Half of each series was sterilized by

[3] Medium used: Dextrose, 40 g.; asparagin, 2 g.; K_2HPO_4, 0.5 g.; distilled water, 1,000 cc.

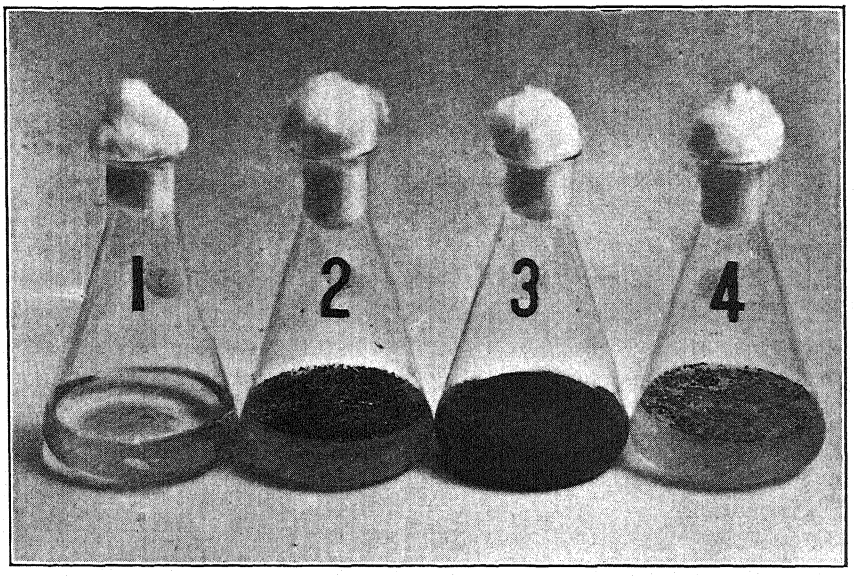


FIG. 1. Culture No. 1—Rhizoctonia inoculated into filtrate from Trichoderma.
" No. 2—Trichoderma " " " " "
" No. 3—Rhizoctonia " " " " Rhizoctonia.
" No. 4—Trichoderma " " " " "

passing through a Seizts filter and the other half by autoclaving for 20 minutes at 15 pounds' pressure. These 4 series were again divided, half of each lot being reinoculated with the organism grown on the media before sterilization and the other half being inoculated with the other organism.

The filter-sterilized series gave the same results as in the first test, that is, no growth occurred in the Trichoderma filtrate inoculated with Rhizoctonia, while normal growth was obtained on all other cultures. In the autoclaved series all cultures grew normally, indicating that the active principle was destroyed by heat sterilization.

In order to determine the thermo-stability of the toxic principle elaborated by the Trichoderma, a series of filtrates from 5-day-old Trichoderma cultures were sterilized with the Seizts filter and then subjected to various heat treatments. Large test tubes, each containing 25 cc. of the filtrate, were held for a period of 10 minutes at temperatures ranging from 50° to 120° C. at 10-degree intervals. After the heat treatment, they were immediately cooled to room temperature and 4 tubes of each series were inoculated with Rhizoctonia and 1 with Trichoderma. All of the Trichoderma cultures showed growth on the second day and grew normally thereafter. The growth of Rhizoctonia, on the other hand, varied according to the heat treatments received by the filtrates. The results are shown in table 3. It

will be noted that no growth of Rhizoctonia occurred in filtrates heated at a temperature lower than 80° C. Heating at 80° for 10 minutes reduced the toxicity of the filtrate appreciably, while heating at 90° left the filtrate only very slightly toxic. In filtrates heated at 100° C. or above, the toxic principle was apparently completely destroyed, as was shown by the fact that Rhizoctonia grew as well in the heated filtrates as in the fresh, unused medium.

The gradual decrease in the toxicity shown by filtrates heated at 70, 80, 90, and 100° C., respectively, may indicate that the toxic principle in the filtrate consists of several products differing in their thermal relationships, as was found by White (8) in filtrates from *Fusarium lycopersici* toxic to tomato plants.

TABLE 3.—*The destruction of the toxic principle in Trichoderma filtrates by means of heat. Growth of Rhizoctonia in filtrates that had been exposed for 10 minutes to various temperatures*

Temperature C.°	Growth	Days delay in growth start	Remarks
Non-heated	None	Infinite	Inoculum died
50	"	"	" "
60	"	"	" "
70	"	"	" "
80	Very slow	5	Mycelium abnormal
90	Slow	1	Finally recovered
100	Luxuriant	0	Growth as good as in fresh nutrient
110	"	0	Growth as good as in fresh nutrient
120	"	0	Growth as good as in fresh nutrient

Destruction of the Active Principle by Aging and Oxidation.—An experiment was undertaken to determine how long the toxic principle would remain active in the Trichoderma filtrate. In this experiment a series of filter-sterilized Trichoderma filtrates were set up in cotton-plugged tubes and inoculated with Rhizoctonia after intervals of 1, 3, 5, 10 and 20 days. No growth appeared in tubes inoculated on the 1st, 3rd, and 5th days. A retarded growth occurred in tubes inoculated on the 10th day and normal growth in those inoculated after 20 days.

Other tubes of sterile Trichoderma filtrates, that had been inoculated with Rhizoctonia 20 days previously and in which no growth had occurred during the time, were reinoculated with Rhizoctonia. These reinoculations

grew normally, indicating that the inoculum first used was killed by the toxic filtrate and not merely held in a state of dormancy. The results of these tests show that the toxic principle in the fresh Trichoderma filtrate is lethal to Rhizoctonia mycelium and that the toxic properties are destroyed in some way when the filtrate is allowed to stand for 10 days or more in cotton-plugged glass containers.

To determine whether the inactivation of the filtrate by aging might be due to oxidation, 2 lots of tubes containing active filtrate were subjected to oxidation by bubbling oxygen through them for 5 and 10 minutes, respectively. These treatments apparently entirely inactivated the filtrates, as was shown by the fact that all tubes so treated gave normal growth of Rhizoctonia, while no growth appeared in control tubes of Trichoderma filtrate not treated with oxygen. The results of this experiment show that the toxic principle in the Trichoderma filtrate is readily destroyed by oxidation and suggest that the lack of toxicity of filtrates that have stood for 10 days or longer may be due to a slow oxidation of the toxic products in the filtrate.

Potency of the Toxic Principle.—In order to determine the approximate strength of the active principle of the Trichoderma filtrate the filter-sterilized medium was diluted with various amounts of water, autoclaved filtrate, or fresh unused culture media, respectively. Five tubes containing 25 cc. of liquid were used for each dilution. Four of these were inoculated with Rhizoctonia and one with Trichoderma. The growth in all of the Trichoderma tubes was normal, regardless of the concentration of the filtrate used, showing, as in former tests, that the Trichoderma filtrate contains no products injurious to the Trichoderma itself. Rhizoctonia, on the other hand, failed to grow unless the filtrates were diluted to a considerable

TABLE 4.—*Potency of toxic principle in filtrate from Trichoderma. Growth of Rhizoctonia in toxic filtrate diluted with various proportions of water, autoclaved filtrate, or fresh nutrient medium*

Ratio toxic filtrate to diluent. Parts by volume		Growth of Rhizoctonia in filtrate diluted with the various diluents		
Toxic filtrate	Diluent	Water as diluent	Autoclaved filtrate	Fresh nutrient medium
5	0	none	none	none
4	1	"	"	"
3	2	"	"	"
2	3	retarded	severely retarded	retarded
1	4	vigorous	slightly retarded	vigorous
0	5	.....	vigorous	"

extent. The dilutions used and the results obtained with the Rhizoctonia cultures are given in table 4.

It will be noted from table 4 that no growth of Rhizoctonia occurred in the Trichoderma filtrates when used full strength or when diluted with approximately equal parts of either water, or deactivated (autoclaved) filtrate or fresh sterile medium. When 2 parts of the filtrate were diluted with 3 parts of the diluent, growth was delayed for several days and in one series was very slow and abnormal for several weeks. Slight retardation in growth also was observed in one series where 1 part of the active filtrate was diluted with 4 parts of the autoclaved filtrate.

It will be noted that both water and fresh nutrient gave similar results when used as diluents. When the Trichoderma filtrate, deactivated by autoclaving, was used, however, there was slightly more retardation in growth. This would suggest that the autoclaving had not completely destroyed all of the toxic materials in the filtrate, despite the fact that apparently normal growth was obtained when the autoclaved filtrate was used directly.

The above dilution experiment was repeated, using water and autoclaved filtrate as diluents, with essentially the same results.

Effect of Toxic Principle on Other Fungi.—In a number of the laboratory experiments discussed above, a few cultures were included in which *Pythium de Baryanum* was inoculated into the Trichoderma filtrate. In every case Pythium proved to be just as sensitive as Rhizoctonia to the toxic products. A few preliminary tests also were conducted with other soil-borne pathogenic fungi but the results from these are not conclusive. It seemed evident, however, that the toxic principle in the Trichoderma filtrate was not equally toxic to all fungi. Some of the fungi grew just as luxuriantly in the active Trichoderma filtrate as in the fresh nutrient.

SUMMARY

Seed decay and damping off of cucumbers induced by Rhizoctonia and Pythium were appreciably reduced by inoculating the soil heavily with a species of Trichoderma. Garden peas showed a similar but less marked response.

Laboratory tests indicate that the Trichoderma may have an antagonistic effect on the growth of Rhizoctonia and Pythium.

The presence of an active principle, toxic or lethal to Rhizoctonia, was demonstrated in a liquid medium in which Trichoderma had grown for 5 days.

The filtrate from a 5-day-old Trichoderma culture was lethal to Rhizoctonia when used full strength or when diluted 40 per cent or less with nontoxic diluents.

The toxic principle in the filtrate was completely inactivated or destroyed by heating for 10 minutes at a temperature of 100° C. The toxicity of the filtrate was greatly reduced by a 10-minute exposure at 90° C. and slightly reduced at 80° C.

The potency of the toxic principle was destroyed by bubbling oxygen through the filtrate for a period of 5 minutes, or by allowing the sterile filtrate to stand in cotton-plugged test tubes at room temperature for a period of 20 days.

Rhizoctonia failed to grow in a freshly prepared filter-sterilized filtrate from a 5-day-old Trichoderma culture, but grew luxuriantly in a similar filtrate after the toxic principle had been inactivated or destroyed by the treatment mentioned above; viz., dilution, heat, exposure to oxygen, exposure to air.

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OBSERVATIONS ON TUBERCULINA MAXIMA, A PARASITE OF CRONARTIUM RIBICOLA

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INTRODUCTION

The purple mold, *Tuberculina maxima* Rostrup (*T. persicina*), has been known as a parasite of *Cronartium ribicola* Fischer for many years in European regions where blister rust is common. Saccardo (9) described this interesting genus in 1880, and in his more recent works (10, 11) he has described the species: *T. persicina, T. maxima* and *T. vinosa* Sacc. Gobi (1), studying a fungus that he called *Tubercularia persicina* Ditm., left the identity of the organism somewhat in doubt. Later, Morini (5), working with *T. vinosa,* made a study of the spores and their process of germination. The latter's work did not agree with Gobi's findings, and there is, therefore, some difficulty in knowing what organisms were used in these early studies.

The species *Tubercularia maxima* was described by Rostrup (8) in 1890 as attacking *Cronartium ribicola* (*Puccinia klebahni*) on *Pinus strobus* in Denmark and Germany. Rostrup separates *T. maxima* from *T. persicina* on slight differences in spore measurements and in color, but the present tendency is to treat *T. persicina* as a synonym.

Tubeuf (14), in 1902, records his observations made on *Tuberculina maxima* and finds the fungus always in direct association with rusts. The spores were found to germinate readily in water, sugar solutions, and acidified gelatin to which fruit juices had been added. Tubeuf states that the spores of this fungus are not produced catenulate in a jelly-like mass but are produced singly by abstriction from the ends of the sporogenous hyphae, and that the air currents are the principal means of dissemination. Tubeuf (15, 16, 17, 18) has been the principal champion in Germany of the use of *T. maxima* as an agency in the control of the white-pine blister rust. Observations and experiments were continued by him until his recent paper (19) appeared in 1930 reporting the successful use of the parasite in greatly reducing or entirely inhibiting the production of aeciospores of the rust in certain regions of Germany.

Lechmere (3) in 1914 gave additional details regarding the life history of the purple mold and concluded that it restricted its attack to the pycnial and aecial structures entirely and did not penetrate the pine host tissues, nor did it attack or destroy the rust mycelium. Spaulding (12), in 1929, and Rohmeder (7), in 1931, report a marked reduction in aeciospore pro-

duction in several regions, including England, Denmark, Belgium, and Germany. Rohmeder's reports cover several areas in the Swabian forest regions.

In this country the first note of the occurrence of *Tuberculina maxima* was made by Weir and Hubert (20) in 1917, recording the presence of this parasite on the native pine rusts in Montana. Since that time the purple mold has been more commonly observed on native rusts throughout the Western United States and Canada. Mielke (4) and others have collected it in fairly large quantities in British Columbia, and, in 1932, R. K. Pierson collected a purple fungus on the rust cankers that he was investigating on the Newman Lake study plots in Washington. This fungus, upon examination, proved to be *T. maxima.* H. N. Putnam, in October, 1932, found a vigorous development of the fungus on the aecial and pycnial pustules of cankers developing upon the test trees planted on the Cheekye experimental plots in British Columbia. He states that the purple mold completely in-

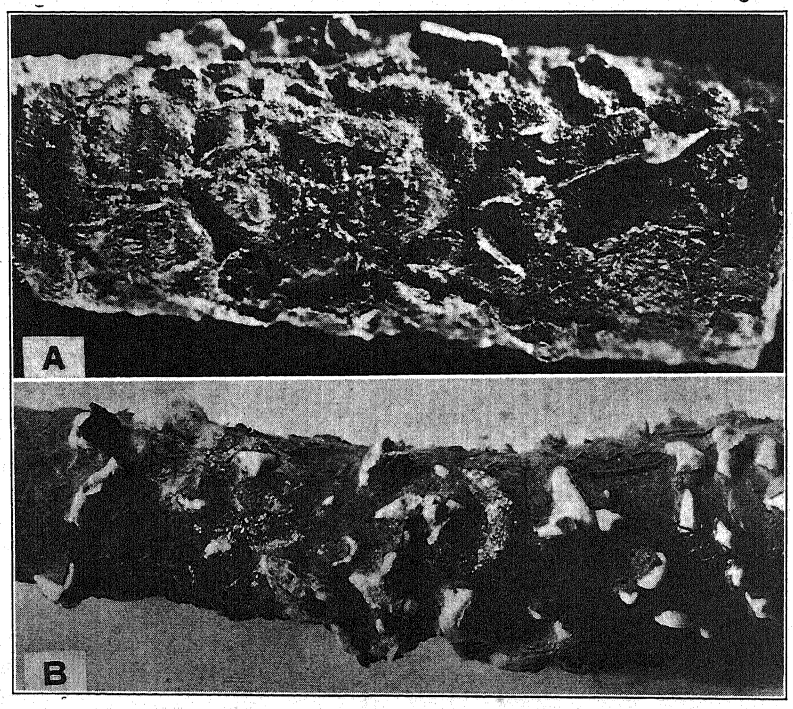


FIG. 1. A. Aecial scars of *Cronartium ribicola* on *Pinus monticola* overgrown by the rust parasite, *Tuberculina maxima.* Note the moldy appearance and absence of peridia. Slightly enlarged. B. Noninfected aecial blisters of *C. ribicola* on *P. monticola,* showing clear-cut outlines and the absence of moldy growth. Normal condition of rust canker.

hibited the aecial development on the parasitized cankers, and that it was found attacking about 1 per cent of the total number of fruiting or aecia-bearing cankers (Fig. 1).

The following is a summary, exclusive of herbaria, of the rust hosts for the purple mold in this country, including the years 1917–1932.

SUMMARY OF RUST HOSTS FOR *T. maxima* IN THE UNITED STATES, THE COLLECTORS AND LOCALITY OF THE COLLECTION

1917.	*Cronartium coleosporioides*	O & I	Weir & Hubert, Montana
1917.	*Cronartium pyriforme*	O & I	Weir & Hubert, Montana
1917.	*Cronartium cerebrum*	O & I	Weir & Hubert, Michigan
1930–31.	*Cronartium ribicola*	O & I	Mielke, *et al.*, B. C.
1932.	*Cronartium ribicola*	O & I	Pierson, Washington
1932.	*Cronartium ribicola*	O & I	Putnam, B. C.
1917.	*Uredinopsis mirabilis*	I	Hubert, Idaho
1932.	*Cronartium coleosporioides*	O & I	Hubert, Idaho
1932.	*Cronartium coleosporioides*	O & I	Chapman, Montana

LIFE HISTORY OF TUBERCULINA MAXIMA

Tuberculina maxima is classified under the Fungi Imperfecti and but one spore stage is known. The conidiospores are large, globose and lilac-colored. A set of 25 spore measurements made of the purple mold developing upon *Cronartium pyriforme* shows the following record: (25) 8.4–13.2 × 12.1–20.9 μ. Av. 10.2 × 12.4 μ. Standard (10 × 18 μ.) The spore measurements given by Saccardo for *T. persicina* are somewhat smaller (7–10 μ) than those usually recorded for *T. maxima*, and Plowright gives 7–14 μ as the range in diameter. Rostrup (8) records the color of *T. persicina* spores as clear violet and states that in contrast to this the spores of *T. maxima* are blue to violet in color. Facts concerning the life history of the purple mold are meager, and it is supposed to attack the spores of its host but not the hyphae. It is not known to attack the bark or wood tissues of the white pines. Observations made in 1917 and since that date, on the habits of the purple mold in attacking *C. pyriforme*, indicate that the fungus develops frequently upon the pycnial stroma beneath the pine epidermis or along the bark cracks. It also sporulates through the pycnial scar openings in the bark tissue. Similar observations have been made regarding its method of developing on *C. ribicola* lesions.

Little seems to be known regarding the overwintering of *Tuberculina maxima*, although some thick-walled spore form would be indicated if it is true that the mycelium develops only in the spore masses and pycnial fluid of the rust fungus. However, it is not improbable that the mycelium may overwinter in the pycnial layer beneath the bark of the host, since examinations made under the microscope of sections cut through pycnial and aecial

areas infected with the purple mold show its mycelium penetrating into the sporogenous layer of the rust fungus. It is reported attacking the uredial stage of rusts but so far it has not been observed on this stage of the blister rust in the Inland Empire or Pacific Northwest regions.

The conidiospores are produced singly from the ends of sporogenous hyphae and form a dense, moldy to powdery mass of purple spores on the subepidermal pycnial stroma, on dried pycnial exudates, pycnial scars and aecial peridia.

The fungus, both the British Columbia strain and the strain obtained from Dr. Tubeuf in Germany, has been tried out on malt agar and a number of other cultural media, but, up to 1933, no successful medium had been found. A study to determine the best artificial media for the rapid development of a large number of spores under laboratory conditions was undertaken by D. J. Stouffer (13) in 1931, but without success. It has, however, been cultured by placing upon malt-agar slants small strips of infected bark bearing the purple mold; but the cultures did not develop independently upon malt agar and soon died. In June, 1933, a pure culture of *Tuberculina maxima,* growing on cherry agar, was received from the Centraalbureau voor Schimmelcultures, Baarn, The Netherlands. This culture was successfully transferred to a malt agar, the agar of which was obtained from Sweden. Sporulation on both agars has taken place, but the spore production, as well as the mycelial development, has been slow. No attempts have yet been made to inoculate blister rust cankers with this strain.

The parasite, more aptly termed the purple mold of rusts, sometimes called the lilac fungus, has been successfully employed in the laboratory as inoculum upon the pycnial stage of the white pine-blister rust, developing on cankers obtained from the Ruby Creek area near Bovill, Idaho.

The spores of this fungus have been repeatedly tested in the laboratory and, in most cases, successfully germinated (Fig. 2) in various solutions by a number of workers. Laboratory studies show that the spores are constricted from the ends of sporogenous hyphae, as reported by other workers. The spores germinate within 12 hours after placing in the culture solutions and produce short, broad, unbranched, hyalin germ tubes. On the third day, in 1 per cent glucose, germination had a tendency to slow up. Branching of the germ tube began to take place on the third and fourth days and by the end of the seventh day the hyphae had attained lengths ranging from 100 to 110 μ. The mycelium is characteristically slow in growth.

RECENT FIELD STUDIES

In 1931 the writer became interested in the fungi found attacking the white-pine blister rust and, on March 30, he wrote to Dr. Tubeuf in Germany for a supply of spore material collected from the *Tuberculina maxima*

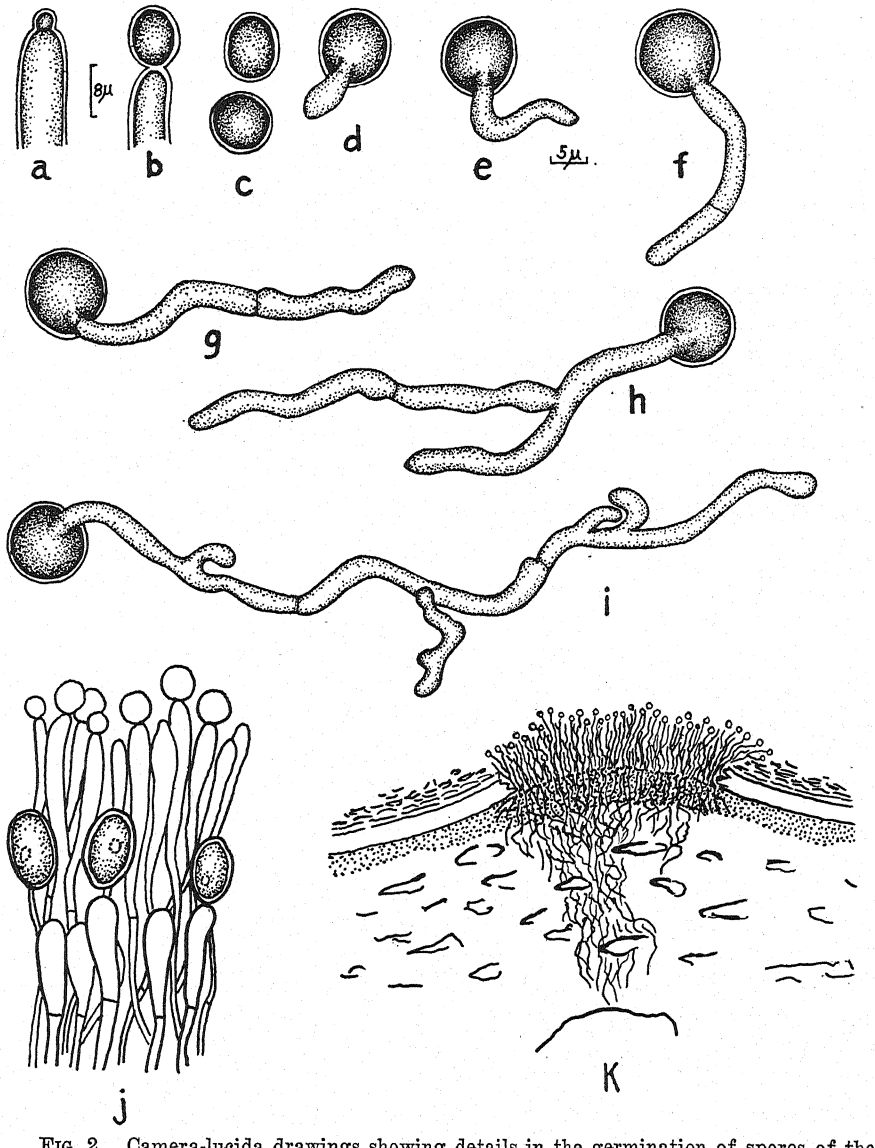


FIG. 2. Camera-lucida drawings showing details in the germination of spores of the purple mold, *Tuberculina maxima*. Germinated at room temperature in hanging drops of 1 per cent glucose solution. a. The end of a sporogenous hypha with a spore in the process of development. b. Nearly mature sport almost severed from the supporting hypha. c. Mature spore (*T. maxima* on *C. coleosporioides*). d, e and f. Germinating spores (*T. maxima* on *C. ribicola*). g. Branched hypha with septa. h. Hypha with swollen segments (*T. maxima* on *C. ribicola*). After 4 days. i. After 7 days. j. Semi-diagrammatical sketch showing the sporogenous layer of the purple mold developing at the surface of the blister rust aecial sporogenous layer. The larger spores are aeciospores. k. Diagrammatical sketch showing the sporogenous layer of the pycnial stage (stippled area) of *C. ribicola* and above it breaking through the bark opening the sporogenous layer of *T. maxima*. Below are shown the hyphal strands of the purple mold extending for a considerable depth into the canker tissue. The blister-rust mycelium is not shown.

infections on blister rust common in that country. Dr. Tubeuf very generously sent a large supply of the spores, which was received in August of the same year. Due to the lateness of the season and to a considerable extent to the extreme dryness of the season, but few field inoculations were made; 12 at Cameron Creek near Elk River, Idaho, and about 10 at Three Bear Creek near the same town. Of the 12 cankers that were inoculated at Cameron Creek 6 were collected in June of the following summer and all of these were dead when cut from the trees. Since a number of the uninoculated cankers also died during this period and subsequent to it, no reliance could be placed upon the results. The inoculations made at Three Bear have produced no results to date.

In 1931 and 1932, collections of the purple mold made at various points in British Columbia were received from J. L. Mielke and T. S. Buchanan of the Division of Forest Pathology and stored in the refrigerator as soon as received. Not all of the last collection, received in August, 1932, was used for field inoculations, but a portion was stored in the refrigerator for

TABLE 1.—*Showing field inoculations made with Tuberculina maxima on blister rust lesions of white pine in Idaho*

Locality	No. of inoculations	Source of purple mold and date collected	Date of inoculations	Number of lesions showing			Results
				Fresh pycnia	1931 aecia	1932 aecia	
Elk River, Idaho Cameron Creek	12	Germany, July, 1931 (Tubeuf)	August, 1931	0	12 old	.....	Negative June 4, 1932 6 cankers dead or dying
Elk River, Idaho Three Bear Creek	10	"	August, 1931	8	10 old	.....	Negative July, 1933
Clarkia, Idaho Middle Fork St. Maries	24 #1–24	Owl Creek, B. C., July 25, 1932	August 3, 1932	21	.....	3	Positive in two cases only. Aug., 1933
Coeur d'Alêne Burnt Cabin Creek	9 #25–33	"	August 27, 1932	9	.....	5	Negative 1933
Oxford R. Sta. Elk Creek, Clearwater, N. F.	4 #34–37	"	August 8, 1932	4	.....	4	Negative July, 1933
Weitas R. Sta. Mouth of Hemlock Creek	13 #38–50	Owl Creek, B. C., August, 24, 1932	Sept. 9, 1932	13	.....	2	Positive in 3 cases only. July, 1933

subsequent study. These collections, fresh from the field and consisting of small thin strips of bark bearing the purple mold and its spores, were used during the summer of 1932 to inoculate a large number of young and old cankers in widely separated regions in northern Idaho. Table 1 gives a summary of the field inoculations made by tieing with heavy thread small pieces of purple molded bark, bark face down, against fresh pycnial exudations or fresh aecial eruptions. All inoculations were labeled with numbered wooden tags fastened to the twigs and branches by means of the fine copper wire attached to them. In some cases the purple spore masses were gently rubbed over the entire surface of the canker.

As the data in Table 1 plainly show, the results of field inoculations produce negative results with the exception of two lesions in the Clarkia area and 3 lesions in the Hemlock Creek area. There are, unquestionably, many factors that are responsible for the weak foothold that the purple mold secured as a result of the 72 inoculations made at various points in Idaho. So little is known regarding the life history of this organism that only a guess can be made as to the most favorable season for applying the spores, the correct stage in the development of the blister rust fungus when these spores are most effective, and the weather conditions most favorable for spore germination and infection. To determine if the spores of the purple mold material were viable, periodic germination tests of the material stored in the refrigerator were made. The spores collected on September, 1932, and stored immediately upon receipt showed good germination, between 8 and 10 per cent on Feb. 4, 1933. On March 17 of the same year, a germination of about 5 per cent was recorded; one or two spores showed germ tubes on April 20 and, when tested on May 20, no germination took place. The spores, under cold-storage conditions at least, are viable for a period of 7 months following their collection in the field.

That the fungus is capable of establishing itself and spreading is evidenced by the infections reported on blister rust cankers at Newman Lake in 1932 and the spread of this infection to neighboring cankers in 1933 and 1934. The spread has been noted also in the British Columbia areas where the purple mold has become established. The difficulty experienced in growing this fungus on artificial media in the laboratory may indicate that it requires rather exacting environmental conditions in nature for its inception and development. We may also be faced with the problem of biological specialization, as Keener has noted for *Darluca filum* (2).

AS A CONTROL AGENCY

Since cross fertilization by pycniospores (6) appears necessary to the copious development of aecia, then an effective biological control might be found in such an agent as *Tuberculina maxima,* which vigorously attacks

the pycnial stage. The pycnial nectar no doubt harbors many fungi. However, little attention has been paid to these fungi and to their probable effect upon the development of the rust. If a natural agent can be found that destroys large numbers of the pycniospores, and by so doing reduces considerably the aecial production, some measure of control may be had at little cost.

In view of the above, it might prove of value to determine the number and kind of insect carriers of pycniospores. A study of their habits may easily lead to utilizing them in carrying to the pycnial drops small quantities of a chemical that would render the pycniospores functionless. In a similar manner, the insect visitors to pycnial stroma doubtless play an important part in conveying fungus spores other than pycniospores from one canker to another.

In Europe, considerable attention has been given to the possible control of the white-pine blister rust by means of the purple mold. The attempts at control on European forest lands were reported to have greatly reduced and in some cases, inhibited the production of aeciospores (15, 3). The few attempts so far conducted in Idaho have proved ineffective and it is obvious, that no matter what measures of destruction any one biological agency may accomplish under favorable conditions locally or in any specific year; the most effective control of the white-pine blister rust will always be accomplished through the destruction of Ribes plants by mechanical pulling, the various types of chemical spraying, the slashing or bulldozing of brush areas, with subsequent burning, and the control of Ribes population by selective cutting of timbered areas.

SUMMARY

The purple mold, *Tuberculina maxima,* reported as an agency in the control of the white-pine blister rust in Europe, is beginning to be studied in this country.

So little is known of the life history of this fungus that attempts made in 1932 and 1933 to spread it among white pines infected with *Cronartium ribicola* in Idaho met with little success. The purple mold has been grown successfully in cultures but its development is slow and spore production is relatively meager. More work will need to be done before quantity production of the spores can be attained for use in control experiments.

Local, natural establishment of the purple mold and its subsequent spread has been noted but the spread has always been limited to small areas. The possibilities of its use as a biological control agency are remote and can in no way compare with the present mechanical and chemical control methods used in the United States.

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INOCULATIONS OF STAGONOSPORA CURTISII ON THE AMARYLLIDACEAE IN CALIFORNIA[1]

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INTRODUCTION

The family Amaryllidaceae contains a number of desirable ornamentals, such as the different forms of Narcissus, Amaryllis, Hippeastrum, Crinum, Leucojum, and other species not so well known. This paper describes some inoculations with *Stagonospora curtisii* (Berk.) Sacc. on these and other species of the Amaryllidaceae grown in California.

THE DISEASE

In a recent paper, Creager (3) has studied the leaf scorch of Narcissus and reports it as having economic importance in the eastern United States. McWhorter and Weiss (9) list the disease in Oregon, while Dougherty (4) and Smith (14) have studied a similar disease in California on Hippeastrum. Other investigators (5, 6, 11, 15) have reported it as present in Europe. Seaver has described (13) *Phyllosticta hymenocallidis* Seaver as attacking *Amaryllis purpurea* Ait. in Louisiana and *Hymenocallis* sp. in Bermuda and in the Greater Bahamas. Petrak (11) describes carmine spots on the leaves of Amaryllis and attributes their cause to *Stagonospora crini* Bubák and Kabát. He records successful inoculation on *Zephyranthes rosea* with *S. crini.* Nicolas and Aggery (10) describe blood red spots on living leaves of *Crinum longifolium* and *C. asiaticum,* and believe them to be caused by *S. crini.*

From the fact that the fungus has been found fruiting on the outer terminal end of the bulb scales, it seems apparent that the leaves may become infected near their tips at the time they are pushing out from the bulb. The disease in California is characterized by red spots on the leaves, flower stems, and sometimes on the petals of *Hippeastrum* sp. The leaf spot is bright red to purple red (blackish red purple).[2] Although small at first, the spots often increase to 50 mm. or more. Pycnidia are apparently developed sparingly in the diseased spots under our climatic conditions, but become more abundant when the diseased leaves are kept in a moist chamber.

On Narcissus no red spots are formed, but the infected areas are brownish and occur on leaves and flower stems. The tissue above and below the

[1] Paper No. 303, University of California Citrus Experiment Station and Graduate School of Tropical Agriculture, Riverside, California.

[2] Ridgway, Robert. Color standards and nomenclature. Washington, D. C., 1912.

infected areas becomes yellow, and the leaf soon dies. The ends of the leaves are the parts most often infected.

In California the disease on Hippeastrum is of only minor importance during seasons of normal rainfall. Some spotting occurs on leaves scattered throughout the field, but no great amount of killing of foliage has been observed. In exceptionally rainy seasons the injury is increased and nearly every plant may have one or more spots. Dougherty,[3] after a careful study of the occurrence of the disease, reported 90 per cent of the plants as being infected with one to several spots. The fungus rarely attacks *Amaryllis belladonna*. The disease is of more importance on Narcissus and may attack and kill plants long before the end of their normal growing season.

SOURCE OF MATERIAL

The cultures used in this study were isolated from stem lesions, leaf spots, and bulb scales of *Hippeastrum* sp., from reddish brown areas on leaves of *Crinum powelli*, from the pycnospores of *Stagonospora curtisii*, and from diseased tissue of Narcissus.

Through the courtesy of H. S. Cunningham, formerly of the Department of Agriculture, Bermuda, the author received some fresh material of *Hymenocallis littoralis* (Jacq.) Salisb., affected with *Phyllosticta hymenocallidis* (13). Some of this diseased tissue was used in inoculating, through wounds, healthy leaves of *Hippeastrum* sp. In the 2 trials made, typical red spots were formed at the point of inoculation, and from this infected tissue a Phyllosticta-like fungus was isolated that was pathogenic on forms of Hippeastrum, Narcissus, and other species of the Amaryllidaceae. (See Table 1, inoculations by cultures from *Hymenocallis* sp.). Attempts to isolate *Phyllosticta hymenocallidis* directly from the diseased leaf tissue of *Hymenocallis littoralis* were unsuccessful, probably because the leaves were too old. The indirect method of using the diseased tissue of *H. littoralis* as an inoculum admittedly leaves some doubt as to whether it is the above fungus or something else in this tissue that causes the infection on Hippeastrum. The remaining plants of Hippeastrum, however, remained free from any natural infection.

THE CAUSAL ORGANISM, ITS SYNONOMY AND DESICCATION

Stagnospora curtisii is the cause of the leaf scorch of Narcissus and the red leaf spot of *Hippeastrum* sp. The fungus is readily isolated by the usual methods and grows well on cornmeal agar, glucose potato agar, and sterilized leaves of Narcissus, Hippeastrum, and Iris. It fruits scantily on the agar media, but pycnidia are abundantly formed on sterilized leaves.

[3] Dougherty, P. I. The red leaf-spot of Hippeastrum. (An unpublished thesis submitted for the degree of Bachelor of Science, Department of Agriculture, University of California, 1914.)

TABLE 1.—*Inoculation on species of the Amaryllidaceae through wounds by spores and mycelium of Stagonospora curtisii*

Hosts inoculated[a]	Sources of cultures used in inoculations[b]				Radius of lesions
	Hippeastrum	*Narcissus*	*Hymenocallis*	*Crinum*	
	Number of lesions				
					mm.
Amaryllis belladonna	3	3	3	2	2–5
**Chlidanthus fragrans*	5	10	6	5	5–10
**Crinum powelli*	3	3	4	7	10–20
**Galanthus* sp.	3	2	4	5	5–10
Hippeastrum vittatum	35	40	5	3	5–50
**Hymenocallis calathina*	2	2	4	2	10–20
**Leucojum vernum*	5	4	11	5	5–15
**Lycoris squamigera*	3	2	3	5	5–20
Narcissus sp.	7	14	2	2	5–40
**Pancratium maritimum*	4	3	6	3	5–10
**Sternbergia lutea*	2	3	3	2	5–20
**Zephyranthus candida*	2	5	3	3	3–10

[a] An asterisk indicates that the species has not before been reported as a host of *Stagonospora curtisii.*

[b] Cultures were isolated from *Hippeastrum* sp., which had been inoculated with tissue of *Hymenocallis littoralis* infected with *Phyllosticta hymenocallidis* Seaver. Cultures were secured from the diseased tissue of species of Hippeastrum, Narcissus, and Crinum.

The spores in artificial cultures are especially variable, as has been noted by Creager (3) and by Smith (14). They are usually small, continuous, or occasionally uniseptate, and could be classified as belonging to the genera Phyllosticta or Phoma. At other times, however, the spores in cultures growing on sterilized leaves of Narcissus or Hippeastrum may be large, 3–5 septate, and typical of *Stagonospora curtisii;* but, even in these cultures, the continuous and uniseptate spores are to be found. The vegetative growth of the fungus may be scant when pycnidia are in great abundance. This variability of spores has been found in all the cultures studied, even though they originated from large, septate spores.

The spores of *Stagonospora curtisii* showed some resistance to desiccation, as shown by the following tests. Dried herbarium material of *S. curtisii* on narcissus leaves was kept in the laboratory at room temperature in paper packets for one year. Dilution cultures made of the spores showed nearly 100 per cent germination. An attempt to germinate the spores from this same herbarium material after 2 years gave negative results. The protection from rapid desiccation of spores is probably largely due to the surrounding pycnidial wall.

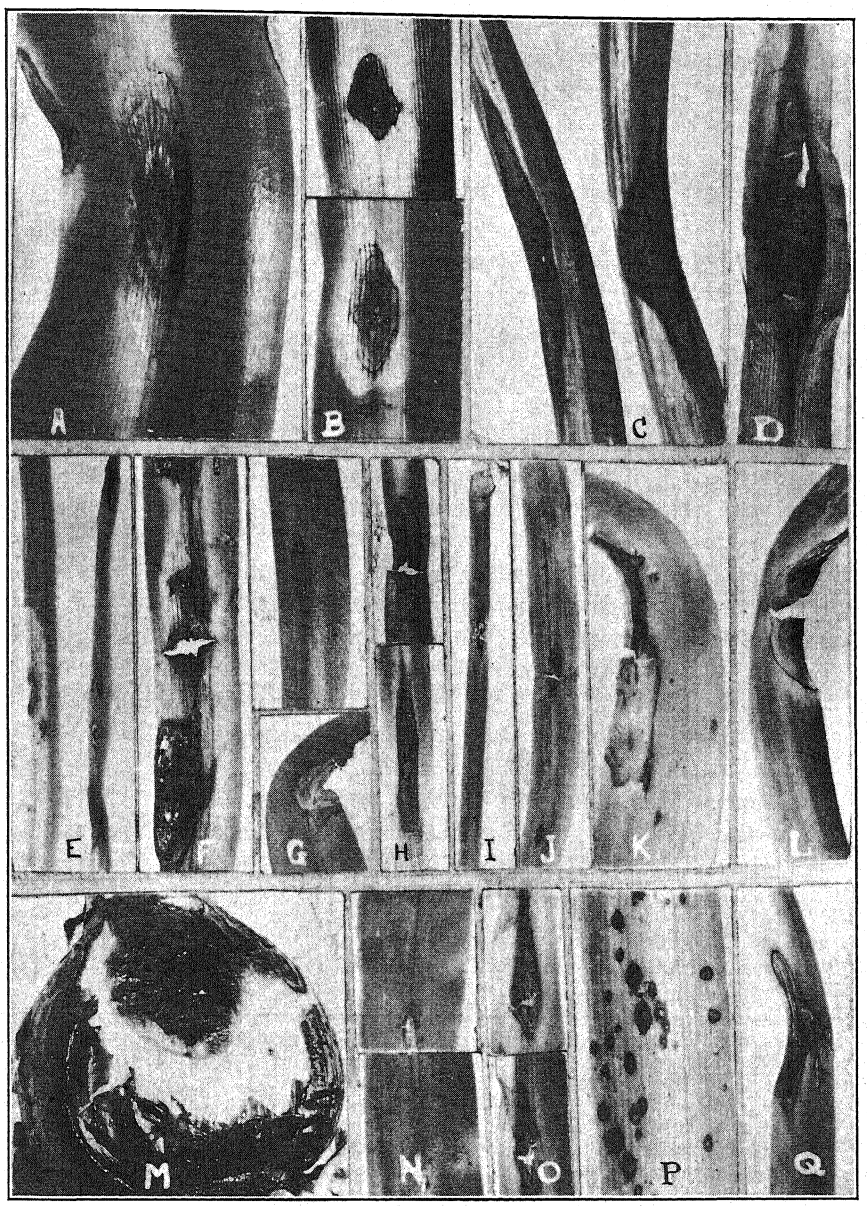


FIG. 1. A. Artificial inoculation on leaf of *Crinum powelli* with culture from *Hippeastrum* sp., infected with diseased tissue from *Hymenocallis littoralis*. Numerous pycnidia on spots. B. Artificial inoculation on leaves of *Hymenocallis calathina* (*Ismene calathina*) through wounds using culture isolated from *Crinum powelli*. Photographed after 5 days. C. Inoculations through wounds on flower stems of *Hippeastrum* sp., using culture isolated from flower stalk of *Hippeastrum* sp. Photographed after 20 days. D. Inoculation on *Pancratium maritimum* by culture from *Hippeastrum* sp., after 15

Narcissus sp., and hybrids of *Hippeastrum vittatum* have long been known to be attacked by a fungus attributed by different workers to Phyllosticta, to Phoma, and to Stagonospora. The names of the following fungi also listed by Creager (3) are believed to be synonyms of *Stagonospora curtisii: Phyllosticta oudemansii* by Saccardo (12), *P. narcissi* by Aderhold (1), *Stagonospora narcissi* by Hollós (6), all occurring on Narcissus; *Phyllosticta* sp. by Dougherty (4), and by Horne and Essig (7), *P. gemmipara* by Zondag (15), *Phoma amaryllis* by Kotthoff and Friedrichs (8), and *Stagonospora* sp. by Petrak (11) on Amaryllis. The writer believes, from his studies, that *Phyllosticta hymenocallidis* by Seaver (13), and *Stagnospora crini* by Bubák and Kabát (2), should be added to this list.

ARTIFICIAL INOCULATIONS ON AMARYLLIDACEAE

Most of the inoculations were through wounds, usually a needle puncture underneath the inoculum, which was prevented from drying out by covering with adhesive tape or Johnson's nurseryman's tape. Inoculations also were made by placing the inoculum in contact with the uninjured tissue and keeping the plant in a moist chamber. The following species of the Amaryllidaceae have been inoculated through wounds:[4] *Amaryllis belladonna* Linn., **Chlidanthus fragrans* Herb., **Crinum powelli* Hort., **Galanthus* sp., *Hippeastrum vittatum* Herb., **Hymenocallis calathina* Nichols (*Ismene calathina* Herb.), **Leucojum vernum* Linn., **Lycoris squamigera* maxim., *Narcissus tazetta* Linn. (Chinese Sacred Lily), **Pancratium maritimum* Linn., **Sternbergia lutea* Ker-Gawl., **Zephyranthes candida* Herb. (Fig. 1.)

While the degree of infection has varied, all the above species have shown positive lesions. The results from atomizing spores on *Narcissus* sp. and on *Hippeastrum* sp. were also positive. The more susceptible are the species of Crinum, Hippeastrum, Hymenocallis, Narcissus, Pancratium,

[4] The species marked with an asterisk are believed to have been hitherto unreported as hosts of *Stagonospora curtisii.*

days. E. Leaves of *Zephyranthes candida* inoculated with culture from *Crinum powelli,* left, and from *Hippeastrum* sp., right. Photographed after 4 days. F. *Lycoris squamigera* inoculated with culture from *Hippeastrum* sp., after 15 days. G. *Galanthus* sp.: Above, inoculated with culture from *Narcissus* sp., after 20 days; below, from *Hippeastrum* sp., after 15 days. H. Inoculation of *Sternbergia lutea:* Below, with culture from *Crinum powelli,* after 20 days; above, from *Narcissus* sp., after 25 days. I. Inoculation on *Chlidanthus fragrans,* after 20 days, with culture from *Narcissus* sp. J. Inoculation on *Pancratium maritimum* with culture from *Narcissus* sp., after 20 days. K. *Hippeastrum* sp. inoculated with culture from *Narcissus* sp. Photographed after 30 days. L. *Lycoris squamigera* inoculated with culture from *Hymenocallis* after 15 days. M. Narcissus bulb inoculated with culture from *Narcissus* sp. Photographed 9 months later. N. *Amaryllis belladonna* inoculated: Above, with culture from *Crinum*; below, from *Narcissus* sp. Lesions small. *A. belladonna* seems to show some resistance. O. *Narcissus* sp. inoculated: Above, with culture from narcissus; below, from Hippeastrum. P. Natural infection on *Hippeastrum* sp. with *Stagonospora curtisii.* Q. Inoculation on *Leucojum vernum* by culture from Hymenocallis after 15 days.

Chlidanthus, Sternbergia, Lycoris, and Leucojum. *Zephyranthes candida,* when infected, developed a small lesion, and the tissue of leaf above and below the lesion became a bright yellow. *Amaryllis belladonna* was very slightly infected (1–2 mm.) when inoculated through a wound. Dougherty also failed to get significant results in his inoculations on *Amaryllis belladonna,* but he was successful in his inoculations of the Amazon lily, *Eucharis grandiflora* Planch, and *Hippeastrum* sp. In the following species of the Liliaceae no lesions developed from inoculations: *Agapanthus umbellatus* L. Her., *Allium neapolitanum* Cyr., *Convallaria majalis* Linn. (lily-of-the-valley), *Hemerocallis aurantiaca* Baker, and *Ixia* sp. Inoculations on *Iris germanica* Linn. were also negative.

SUMMARY

A disease of Narcissus and Hippeastrum caused by *Stagonospora curtisii* is described as occurring in California. It was found to be pathogenic on the following species of the family Amaryllidaceae: *Amaryllis belladonna, Chlidanthus fragrans, Crinum powelli, Galanthus* sp., *Hippeastrum* sp., *Hymenocallis calathina, Leucojum vernum, Lycoris squamigera, Narcissus* sp., *Pancratium maritimum, Sternbergia lutea, Zephyranthes candida.* The spores are variable in size, shape, and septation. In the same pycndium may be found large, 1–5 septate spores and smaller, continuous ones, although in cultures nearly all of the spores may be continuous. Apparently the pycnidium can protect the spores for some time from desiccation. In some tests of dry herbarium material, spores were viable after one year.

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MACROSPORIUM AND COLLETOTRICHUM ROTS OF TURNIP ROOTS

CHARLES CHUPP

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Macrosporium herculeum E. and M.

In the early spring of 1932 specimens of diseased turnips were received from Columbus, Ohio, with the statement that the trouble affecting them was important in that part of the State. It had been observed first on the foliage of the crop during the warm, moist weather of the previous August. Later, a majority of the leaves dropped except a small central green cluster. Simultaneously with leaf spotting the roots became affected, so that many plants died and rotted completely. The remaining roots grew no larger and were unmarketable at the end of the season. Later planted turnips became affected also, but to a much less degree. When the roots of this late crop were placed in pits, the high winter temperature apparently favored the growth of the fungus, for most of the crop thus stored was destroyed.

The specimens sent to the laboratory showed circular lesions, usually consisting of two or more zones. The central part of each spot was com-

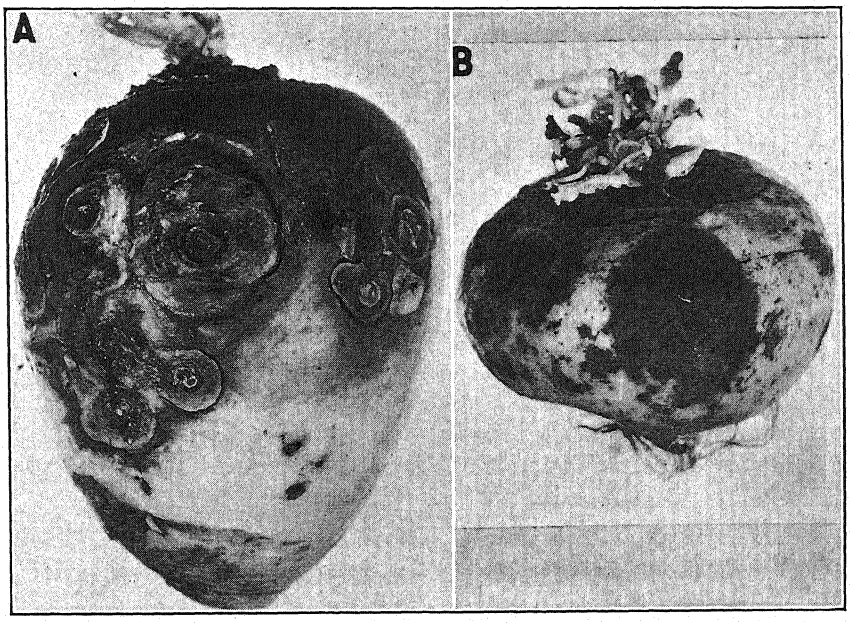


FIG. 1. A. Lesions caused by *Macrosporium herculeum* on field turnip. B. Turnip root 20 days after inoculation with *M. herculeum*.

posed of rather dark brown tissue or was whitened by aerial growth of mycelium. This area was bounded by a narrow dark band (Fig. 1, A). Lesions varied from no band to several, and from one uniform brown shade to several shades and sometimes even almost black. The spots never seemed to be depressed nor the affected tissues watery.

In isolations, the first plantings in nearly every case gave pure cultures of Macrosporium. These cultures were abundantly supplied with conidia, the shape and size of which compared closely enough with the illustrations and measurements given by Weimer (5) and earlier workers (Stewart 3, Ellis and Martin 1) to consider the organism identical with *Macrosporium herculeum* E. and M. (or Alternaria for those who divide the genus). Weimer's drawings show slightly thicker beaks, as compared with the width of the spore body, than do those from the Ohio material (Fig. 2, D). His measurements are $16–28 \times 125–225\ \mu$; spores from naturally infected turnips and from cultures measured $11.4–24.7 \times 79.8–342.0\ \mu$, with an average of $17.5 \times 143.6\ \mu$ for a hundred spores.

Since the fungus had not been reported as causing serious root trouble, inoculations were made on leaves and roots with spore suspensions, and into roots with mycelium. Leaves uninjured by pricking showed infection before injured ones. Visible infection was present on the leaves eight days after inoculation (Fig. 2, A). Under moist greenhouse conditions the parts between the spots soon withered, and the whole leaf died. The Macrosporium fruited slightly on these spots and the conidia were identical with those from the original specimen. Reisolations from these spots also were made with ease.

A spore suspension dropped on turnip roots in moist chambers or bits of mycelium from agar laid on the uninjured root surface produced infection very slowly under laboratory conditions. But when the epidermis was injured with a scalpel and the inoculum placed against the exposed tissue, one hundred per cent infection was obtained, while checks remained healthy. Figure 1, B shows a turnip twenty days after inoculation. Under moist-chamber conditions the lesion is uniformly dark brown, slightly sunken, and wetter in appearance than lesions caused by natural infection. In such an infected root, cut open, the rot extends almost to its center, is uniformly brown throughout the invaded tissue, and at its edges gradually blends with the white of the normal area. The rot is darker colored than that caused by *Erwinia carotovora* and is not so soft in texture.

Turnip seeds, as represented by varieties and strains bought in the open market, almost uniformly show the presence of *Macrosporium brassicae* Berk. (Alternaria) when plated on agar. An occasional lot of seed may show *Macrosporium herculeum,* but apparently never in large amounts and then mixed with the former species. The two species can readily be told

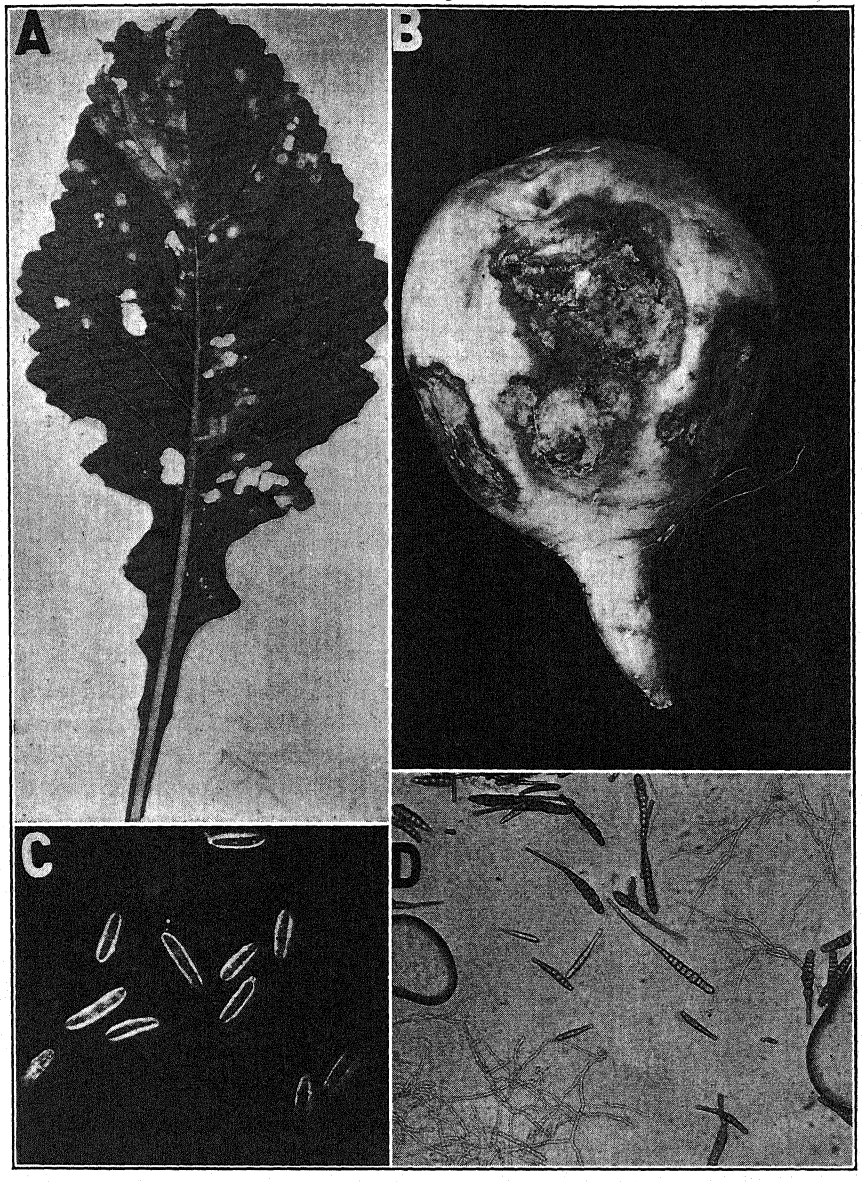


FIG. 2. A. Leaf 8 days after inoculation with Macrosporium from diseased turnip roots. B. Turnip root naturally infected with *Colletotrichum higginsianum*. C. Spores of *C. higginsianum* from turnip root. ×500. D. Spores of *M. herculeum*. ×60.

apart, as shown by Weimer's drawings (b, c). Preliminary tests showed the two species to be approximately equal in their resistance to heat and fungicides. Therefore, in the following seed-treatment experiments, the *Macrosporium* infected seed counts were made without attempting to determine the relative species numbers. The seeds used in the experiment showed the presence of both fungi.

TABLE 1.—*Effects of various seed treatments in reducing the number of seeds producing Macrosporium colonies on agar plates*

No. of seeds plated	Treatment	Length of treatment	No. showing Macrosporium growth
108	Calcium hypochlorite, decanted solution	2 hrs.	62
109	" " " "	2.5 hrs.	15
55	" " " "	1.5 hrs.	8
157	Soaked in water 20 hrs., treated in $HgCl_2$–1–1000, then rinsed in 70% alcohol	3 min.	46
111	$HgCl_2$–1–1000 and rinsed in 70% alcohol	25 min.	28
1706	Hot water 50° C.	10 min.	1

Check plates, without treatment of any kind, were contaminated with so many organisms that it was impossible to make counts of the Macrosporium infected seeds. Apparently hypochlorite and corrosive sublimate could not be depended upon for controlling the fungus, possibly due to the fact that the mycelium grows under the seed coat, although not observed to penetrate farther into the seed, for, when portions of the naked seed were macerated and stained, no mycelium could be found. Furthermore, if seed is soaked until the seed coats can be slipped off, the naked seed treated for 3 minutes in corrosive sublimate and rinsed in 70 per cent alcohol before plating on agar, no growth of Macrosporium appears on the media.

The Macrosporium is very susceptible to injury from hot water. In preliminary experiments three-minute treatments at 50° C. apparently killed the fungus in a majority of the seeds. This short treatment, however, does not kill the surface bacteria, so that Petri-dish platings were too contaminated to make accurate counts.

No opportunity was available for studying the effect of hot water seed treatment on field control of the disease.

Colletotrichum higginsianum Sacc.

In March, 1932, specimens of diseased turnips were received from Rochester, N. Y. These cold-storage turnips had been recently washed and

packed for market. They were of the elongated white type and on each were two to five lesions.

The rot begins on the roots as a small, dry sunken point, and as it enlarges, the affected tissue becomes dirty grayish or light tan in color. The larger the spot becomes, the more sunken the tissue, the margin being abruptly depressed and fairly regular (Fig. 2, B). The older spots may have a slightly wrinkled margin, particularly if bacterial soft rot follows the Colletotrichum. The bacteria apparently gain entrance at the margin of the lesion and soon encircle it with a light- to tobacco-brown, wet-appearing rot, which finally may include the whole root. Penicillium may grow over the dry lesion, but it is very noticeable that it does not extend into the tissue thoroughly invaded by the bacteria.

If an inoculated root is placed in a moist chamber, the infected part is sparingly covered with aerial mycelium. No fruiting bodies are visible to the unaided eye, but in mounts of mycelium scraped from the root, a large number of Colletotrichum conidia are found (Fig. 2, C).

These conidia compare very closely in size to those described by Higgins and to those of the fungus named *Colletotrichum higginsianum* by Saccardo, the measurements being $2.5–4 \times 15–20\ \mu$ with an average of $3.2 \times 18.1\ \mu$ for the conidia of the Colletotrichum on turnip, while Saccardo records $3–3.3 \times 15–17\ \mu$, and Higgins $4–5.5 \times 13.5–19.5\ \mu$ for those of the fungus discovered in Georgia. They are perfectly hyaline, cylindrical, with rounded ends, and occasionally with a marking that has the appearance of a cross wall.

A suspension of these spores sprayed on a turnip leaf, or placed on the exposed tissue of the turnip root, infects readily, producing lesions on the foliage similar to those on the type material of *C. higginsianum*, which Higgins sent for comparison, and on the roots lesions similar to those found on naturally infected turnips.

Higgins, in his publication regarding the fungus (2), makes the statement that " . . . during two previous seasons the disease had been very destructive to young turnip (leaves), but observations on specimens sent from this field indicate that the greater part of damage was probably due to a bacterial soft rot of the roots." He may possibly have found only the late stages of the Colletotrichum disease after the soft-rot bacterium had overrun the original lesions. On the other hand, this leaf-invading organism possibly affects the roots only under the most favorable condition and therefore is not often present in the field.

The fungus overwinters in partly rotted leaves kept moist among old leaves on the soil and was successfully reisolated from them. No trace of it, however, could be found on or in the seeds, even though numerous plantings were made of disinfected whole seeds and seed coats, as well as coty-

ledons and embryos that had been removed from their outer covering. Unfortunately none of the seed from the stock that grew the diseased crop was available for experimentation, so that the negative results obtained in the attempted isolations may indicate merely that the seed strains available were not infected.

SUMMARY

Macrosporium herculeum and *Colletotrichum higginsianum*, both previously listed as leaf parasites on various cruciferous crops, are shown to cause serious turnip root rots either in the field or in storage.

The Macrosporium is carried on or in the seed and can be killed by seed treatment for 10 minutes in water at 122° F.

The Colletotrichum could not be proved to be carried on or in the seed. The fungus, however, does overwinter in old affected leaves.

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LITTLE LEAF OR ROSETTE OF FRUIT TREES IN CALIFORNIA

ANTONI KOZLOWSKI

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Shoots of peach, apple and plum trees, producing abnormally small leaves and finally destroyed by an exanthema, were investigated in the summer of 1933. From the bark parenchyma of the last season's shoots Monilia was isolated.

Some preliminary experiments on the infection were carried out in August, 1933, and showed positive results.

Twigs with small leaves were marked on the sick trees in the orchards concerned in the autumn of 1933, and were examined in the winter of 1934. The presence of Monilia in the bark of the shoots was established and there is no doubt that Monilia causes a fatal injury of the trees concerned. The infection of the trees is facilitated by the unfavorable environmental conditions, as for instance by the anaerobic soil conditions in the peach and apple orchards in Delhi, Merced County, and in Sebastopol, Sonoma County, respectively, or by sand drought in the plum orchard in Bakersfield, Kern County. In this latter orchard the Japanese plum trees grow in deep sand. The reaction of the soil solution is about pH 8.4 to pH 8.6; there is a deficiency of potassium, nitrates and phosphates at the depth of 3 to 6 feet. The trees are exposed to a severe drought during warm, rainless weather. The shoots collected at the beginning of March of 1934 showed that their winter buds were entirely dry and fell off readily; no parasites could be found in the buds thus injured. The main and the lateral small shoots were decayed at their tips. The scars from the fallen leaves and buds were not healed. On cutting off the epidermis at the base of the scars brown spots could be noticed, which represented decayed fibrovascular bundles connecting the leaf petiole with the stem. Such lesions could be detected at the base of almost all scars of the leaves. In a majority of cases a pure culture of Monilia was isolated.

The soil in the apple orchard investigated is a sandy loam; the reaction of the soil solution was a pH 5.7 to 4.8. There was a deficiency of phosphates at the depth of 2 to 6 feet. Only traces of nitrates were found at the depth of 3 feet. Processes of anaerobic fermentation were found at the depth of 4 feet during winter. A clay subsoil was found at the depth of 5 feet. The apple shoots collected at the end of January, 1934, showed that the meristematic tissues in the terminal buds were entirely destroyed. It

looked as if the meristematic cone were cut off at its base so that the medulla was exposed to the surface. The lesion was covered with hairs; the medulla contained healthy greenish cells with compound crystals of calcium oxalate and showed no infection. The scales of the terminal buds were peeled off so that only a few were left and even these were dstroyed at their tips. The lateral winter buds did not show any injury of their meristematic tissues, which, however, were poorly developed. The hairs of the scales of the terminal buds were covered with hyphae and conidia of Monilia. This fungus lives at first saprophytically on the scales of the buds and gradually penetrates into the bark of the shoots.

Among the hairs of the scales of the terminal buds was frequently found a microscopically small snout mite, apparently a species intermediate between *Notophallus* sp. (Eupopidae), and *Bdella* sp. Its rostrum is thick and shorter than that of Bdella, and shows a long, brown, meridianal line. The mite is light brown, almost transparent, and has a few brown spots at the hind part of its dorsum. Its legs terminate in two simple claws. The palpi are biforked. A few bristles cover the dorsum and the legs. It is possible that this mite is not purely predaceous and may cause the above described destruction of the terminal buds.

The soil in the peach orchard investigated is a slightly loamy sand. The reaction of the soil solution was pH 7.3 to 8.4. Hardpan was found at the depth of 6 feet. Processes of anaerobic fermentation were found at the depth of 5 feet. An accumulation of sodium chloride was found at the depth of 4 feet. The shoots of peach trees collected at the beginning of March, 1934, were in a majority of cases entirely healthy. The winter buds showed no injury. In a few cases, however, brown spots were found in the bark parenchyma just below and on both sides of a bud. From such lesions *Monilia* sp. was isolated and found to be identical with the species isolated last summer from the last season's twigs. The spores of that fungus, although not numerous, were found on the surface of the winter buds.

The infection of the twigs of peach trees seems to progress much more slowly than that of plum trees.

On some shoots an injury of the bark was caused by insects and there were found eggs of the California katydid, *Scudderia furcifera,* attached in rows to the twigs.

The species of Monilia, isolated from the above 3 kinds of trees, seem to be very closely correlated and greatly resemble *Monilia cinerea.*

RESULTS OBTAINED FROM MORE RECENT EXPERIMENTS

The formation of abnormally small leaves in diseased peach, apple, and plum trees studied in California apparently is due to the following 3 factors: anaerobic soil conditions, infection by *Monilia* sp., and climate.

Anaerobic soil conditions found in the orchards of peach and apple trees under investigation, which were grown in a sandy soil with a faulty substratum, hardpan and clay subsoil, respectively, caused serious injury to the roots. The leaves of the trees exposed to such conditions were of normal structure and texture but of subnormal size; they also were chlorotic and mottled when the soil reaction was alkaline. The trees so affected were readily invaded by *Monilia* sp., which caused the leaves of the infected shoots to be small, stiff, and of glassy appearance; they grew in clusters. An infection by *Monilia* sp. was mainly responsible for the little-leaf formation on the plum trees grown in a deep sand, in which the trees were exposed not to anaerobic soil conditions but rather to a severe sand drought.

A formation of a witches'-broom-like growth of the shoots is possibly due not to Monilia but to some other fungus.

A formation of small leaves under the influence of anaerobic soil conditions can be readily reproduced experimentally, above all when the meristematic tissues are exposed to such injury during the rest period of the trees, as may be seen from the following instance: Small apple trees were grown about 18 months in 1-gal. crocks containing a loamy sand that was quite rich in iron oxide and iron hydrate. In one series, the soil was oversaturated with water in the first week of December, and, in another series, during the first week of January. A layer of water about 1 cm. deep covered the soil and became more or less rusty. The water was allowed to evaporate spontaneously. The trees started to develop their leaves in mid-February; the leaves in the first series were small and died in March; those in the second series were small, $\frac{1}{2}$ to 1 cm. long, during March, but they began to increase in size towards the end of April. The soil was kept only slightly moist during the second part of February and the following months. Apparently, the cessation of the anaerobic conditions caused an improvement of the trees in the second series of experiments. The leaves in the controls were 5 to 10 times larger than those in the second series in March and April.

An artificial formation of small leaves during the summer months could be induced by an addition of 300 cc. of a culture medium of the nitrate-reducing Pseudomonas to one gallon of the sand in which the small peach trees had been grown for a few months. The leaves of the trees thus treated fell off in about 10 days; the new ones, formed about 6 weeks later, were very small and the plants died in 2 months.

An experimental reproduction of the formation of small leaves under the influence of an infection by *Monilia* sp. has been attempted with some positive results. This problem, however, demands special investigation with a reproduction of all environmental conditions found in the orchards concerned.

It is very possible that by proper regulation of the anaerobic conditions of the soil one could inhibit the growth of the leaves during the entire summer season.

In conclusion, the author wishes to express his thanks to J. C. Martin and R. O. Overstreet for the soil analysis; to N. D. Hudson, the Assistant County Agent in Bakersfield, Kern Co.; to Enoch Torpen, the Assistant County Agent in Santa Rosa, Sonoma Co., to E. C. Ruehl in Delhi, Merced County, for their cooperation in securing the soil samples and in marking and collecting the shoots of the trees, and to Mr. Pederson for his very kind help in the greenhouse.

PHYTOPATHOLOGICAL NOTES

A Portable Rust-Inoculation Chamber.—In the conduct of field nursery experiments at the Arlington Experiment Farm, Rosslyn, Va., involving a study of host reaction to cereal rusts, it often is desirable to inoculate plants grown from seed obtained from specific head selections, or a limited number of plants from one or more crosses, under highly favorable conditions for rust infection. To accomplish this object, the writers designed a portable compo-board chamber, 8 ft. x 4 ft. x 4 ft., equipped with a cover, 8 ft. x 4 ft. in dimensions.

When the nursery row, or part of a row, selected for inoculation has reached the desired stage of plant development, the chamber is placed over it and pressed down until the lower edge is snugly in contact with the soil at all points. It is then banked with soil to insure retention of humidity within the chamber and prevent a too hasty dissipation of the artificial dew subsequently to be applied by means of an atomized spray of tap water. After the plants are generously sprayed, they are liberally dusted with urediospores mixed with some innocuous white dust, such as powdered talc. The talc powder increases the visibility of the inoculum and thus insures a more uniform distribution of the spores without in the least affecting their germinability or potential capacity to produce infection.

To obtain the maximum infection, the inoculum should be applied in late afternoon, provided the day be warm and sunny. If the sky be overcast,

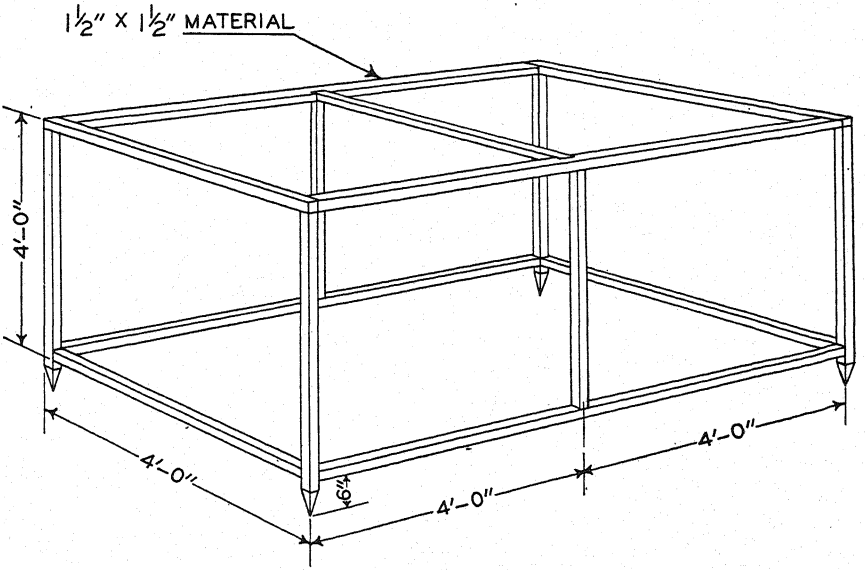


FIG. 1. Working drawing of rust-inoculation chamber showing details of construction of the frame.

the spore shower may be administered earlier in the day. The cover of the chamber is then put in place and the spores are allowed to incubate 24 hours or longer, when the cover is removed and the chamber is reset for a repetition of the operation. Its lightness renders it easily portable by two men.

By means of the inoculation chamber and inoculation technique here described, the writers have been able to subject to individual test in the field a large number of selections and varieties of oats (Fig. 2), under meteorologic conditions quite inimical to the natural development and spread of infection. Moreover, it has enabled them to subject these selections and varieties to infection by known physiologic forms of crown rust (*Puccinia coronata*) and stem rust (*P. graminis avenae*) of oats.

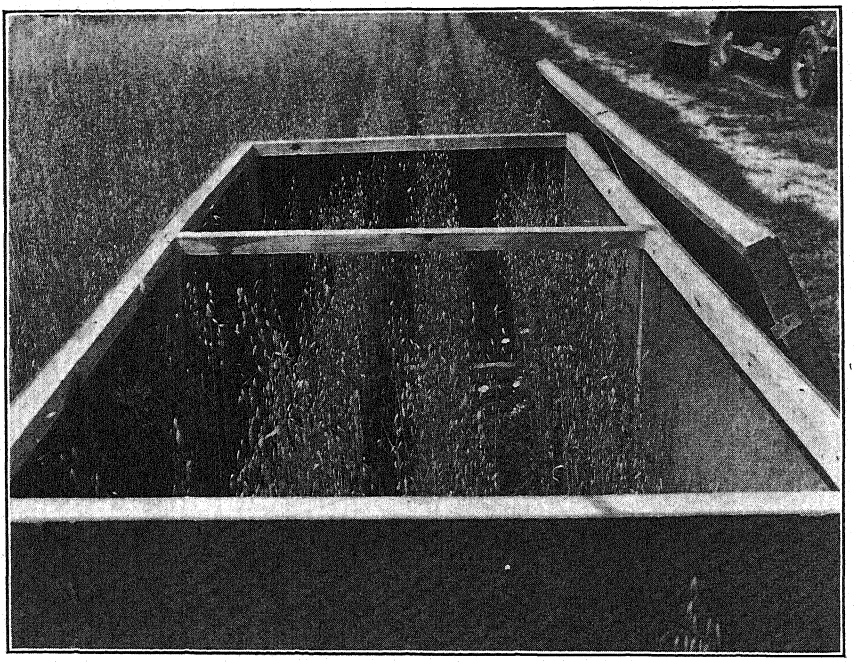


FIG. 2. Inoculation chamber, with cover removed, showing rust-inoculated portions of four nursery rows of oats.

Following is a description of the inoculation chamber and specifications for its construction.

Specifications

This inoculation chamber consists of a rectangular compo-board box covered by a lid of the same material. The frame for supporting the compo-board is made of dressed pine, approximately 1½ in. square, put to-

gether with No. 12 casing nails. The compo-board is attached to the frame with shingle nails, its smooth surface outside (Fig. 2).

The box is 8 ft. long, 4 ft. wide, and 4 ft. high to the cover. The cover is 8 ft. long by 4 ft. wide.

Frames for the ends are made first. The legs or corner posts are $4\frac{1}{2}$ ft. long, sharpened at the lower end to facilitate pressing them into the soil and thus insuring stability of the chamber in the event of a windstorm. The upper or top crosspiece for each end is nailed on top of the legs, and the lower crosspiece of each end between them so that the distance between these 2 crosspieces is approximately $3\frac{3}{4}$ ft. and the over-all distance is 4 ft. The compo-board is then nailed to each end frame. After the ends are completed, they are connected by 4 longitudinal supports, 1 each at the top and bottom on each side. These supports are $7\frac{3}{8}$ ft. long and are toe-nailed into the upright corner posts, their outer edges being kept flush with those of the top and bottom crosswise end braces of each end frame.

To further strengthen the chamber an upright brace is nailed between the longitudinal supports midway on either side and a third brace is nailed crosswise of the box midway between the 2 top longitudinal supports. The compo-board sides are then nailed in place so as to cover the edges of the pieces of compo-board covering the ends.

The cover is made of one piece of compo-board, 8 x 4 ft., attached to an oblong pine frame, 4 x 8 ft., outside dimensions. This frame is made of $1\frac{1}{2}$ in. dressed pine. To add strength to the cover, a brace is placed across it midway between the ends. To secure the cover against disturbance by wind, a hook is placed outside the box on either side near the bottom of the upright middle brace and a clothesline rope is then drawn over the box and tied to these hooks.

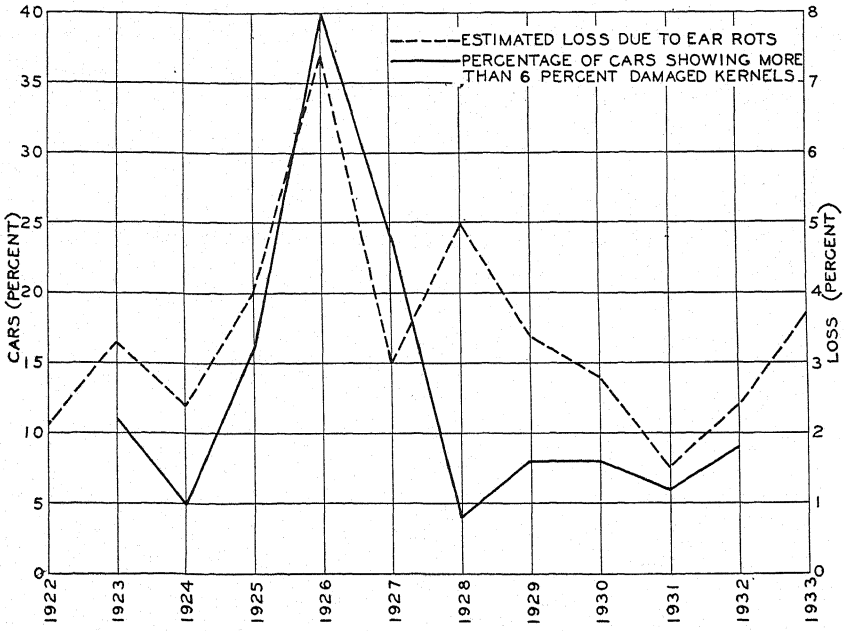
If the soil be too hard, it sometimes is necessary to loosen it with a spade in order that the corner posts may be pushed into the ground. A few taps with a maul on the top of each corner post will settle the chamber to a position of complete contact with the soil. Any openings under the bottom of the chamber can be remedied with a spade so as to stop any vents.—H. B. HUMPHREY and F. A. COFFMAN, Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

Losses From Corn Ear Rots in the United States.—In an earlier paper[1] attention was called to certain of the estimates of losses from plant diseases in the United States compiled by the Plant Disease Survey that seemed especially worthy of consideration. In two of these cases the probable accu-

[1] Stevens, Neil E. Some significant estimates of losses from plant diseases in the United States. Phytopath. 23: 975–984. 1933.

racy of the Survey estimates was attested by their close correspondence with some other method of measuring disease losses.

A third instance of this appears in the estimates recently compiled by the Survey of losses from corn ear rots from 1922 to 1933 shown in figure 1. Information as to losses from corn ear rots, over aproximately the same period but derived from a wholly independent and quite different source, is also given in figure 1.[2] This information, compiled by the Grain Division of the Bureau of Agricultural Economics, gives the annual variation in "damaged kernels" expressed as percentage of cars showing more than 6 per cent total damage in the check inspections made by over 40 offices of Federal Grain Supervision. All observers are agreed that ear and



—— Percentage of cars showing more than 6 per cent damaged kernels.
--- Estimated loss due to ear rots.

FIG. 1. Percentage of cars of corn, showing more than 6 per cent damaged kernels, as indicated by reports of Federal grain inspectors of the Bureau of Agricultural Economics, for the years 1923 to 1932, inclusive. (After graph published by Grain Standards Educational Committee.[2])

Estimated losses in corn due to ear rots for the United States as a whole, and compiled from reports from collaborators of the Plant Disease Survey.

[2] Corn: Annual variation in the principal factors grading receipts lower than No. 3, Nov. 1, 1923, to Oct. 31, 1932. Graph on page 10 of Grain Inspector's Letter. Issued from General Field Headquarters Grain Division, Bureau of Agricultural Economics, Department of Agriculture, by the Grain Standards Educational Committee. Dec. 19, 1932.

kernel rots are very largely responsible for the damaged corn included in the grade factor "total damage."[3]

The correspondence of the major features of these two curves, one based wholly on field observations and the other on inspections at terminal markets, is obvious. Both show a low point in 1924, followed in 1926 by the largest losses of the period, and relatively much smaller losses in the last 4 years, with a low point in 1931. The chief disparity between the curves appears in 1928.

The close agreement between these two sets of figures, derived from such totally unrelated sources, strengthens the probability that both give fairly accurate pictures of the major fluctuations in losses from corn ear rots in the United States.—NEIL E. STEVENS and JESSIE I. WOOD, Bureau of Plant Industry, Washington, D. C.

Joined Spores of Ascochyta viciae.[1]—In an examination of pycnidia of *Ascochyta viciae* Lib., produced on autoclaved wheat straw, it was observed repeatedly that the spores exuded from them in a mass and did not separate freely in water. Vigorous pressure applied to the cover slip so as to flatten the spore masses failed to effect their separation. They were found to be held together by short tubes, about 2 μ long, and of the same diameter as the germ tubes. At first, it appeared as though a spore had developed a short germ tube, which in turn had given rise to a second normal pycnospore. If such a process were repeated successively, it is possible that a chain or group of 15 or 20 joined spores could be formed. However, the spore masses were observed in pycnidia within 3 days after their appearance and within 13 days after subplantation; so it would seem that a theory of successive spore germination could not account for this phenomenon. It is considered more likely that the spores were formed in this condition from the basal cells or conidiophores of the pycnidium.

The tubes showed variation as to their points of attachment and the spores were connected according to no definite pattern in the following manners: (1) from region of septum to a similar location of another spore, (2) from distal end to distal end of a second spore, and (3) from region of septum of one spore to distal end of another spore. For this reason a compact mass rather than a long chain of spores was formed.

Cultures up to the age of 10 weeks were examined and still showed some of these spore unions, although in lesser amounts than did the younger cultures. Growths on oat and rye straw, arising from the same strain of

[3] Holbert, J. R. Plant breeders make progress in developing disease-resistant corn. U. S. Dept. Agr. Yearbook 1934: 285–287. 1934.

[1] Approved by the Director of the New York State Agricultural Experiment Station for publication as Journal Paper No. 48.

Ascochyta viciae (N. Y. No. 107) and developed under an identical environment, contained only normal free pycnospores. Moreover subplants of this strain cultured on wheat straws from other fields developed only an occasional spore union.

In the expectation that this phenomenon would be observed again the original wheat-straw medium was reautoclaved and subplantation was effected with strain N. Y. No. 93. Fungal growths developed promptly and pycnidia formed profusely. A few of these contained the described joined spores but by no means as plentifully as in the first mentioned. Seven strains of *Ascochyta viciae* recently isolated from imported vetch seeds were cultured on autoclaved wheat straw from several fields. A careful examination of 500 pycnidia did not reveal any spore unions. The same strains were cultured also on autoclaved stems of white-blossom sweet clover, winter vetch, white kidney bean, yellow trefoil, and autoclaved oat and rye straw. No joined spores were observed in the resultant pycnidia.—WILLARD CROSIER, New York State Agricultural Experiment Station, Geneva, N. Y.

Nuclear Phenomena in Helminthosporium gramineum.—Recently, Christensen and Graham[1] demonstrated that *Helminthosporium gramineum* Rab. comprises numerous cultural and parasitic races. They found also that new races arose frequently in culture either as sectors or "patch" variants, as well as in or on the living host. As an aid to the elucidation of the nature of variation in this species, the writer has made cytologic studies of various growth stages of the pathogen.

Of the various killing and fixing solutions used, Flemming's weaker was one of the most satisfactory. Iron-alum haematoxylin was used for staining.

The mycelium produced in artificial culture was stained and examined. In addition, preparations were made from seed coats and endosperms of seed infected as a result of artificial inoculation, from diseased seedlings before the emergence of the young leaves from the coleoptile, and from mature diseased leaves. The cells of the hyphae were almost universally multinucleate (Fig. 1, B). Macroconidia and microconidia, which were occasionally produced in chains from the macroconidia, also were multinucleate, each cell usually containing 4 to 7 nuclei, although the range in number was from 1 to 13. The germ tubes are, of course, also distinctly multinucleate.

The origin of the several nuclei in a cell is an important consideration in surmises on their possible effect in variation in the fungus. Observations indicate that they probably were not derived from a single original nucleus.

[1] Christensen, J. J., and T. W. Graham. Physiologic specialization and variation in *Helminthosporium gramineum* Rab. Minn. Agr. Exp. Sta. Tech. Bul. 95, 1934.

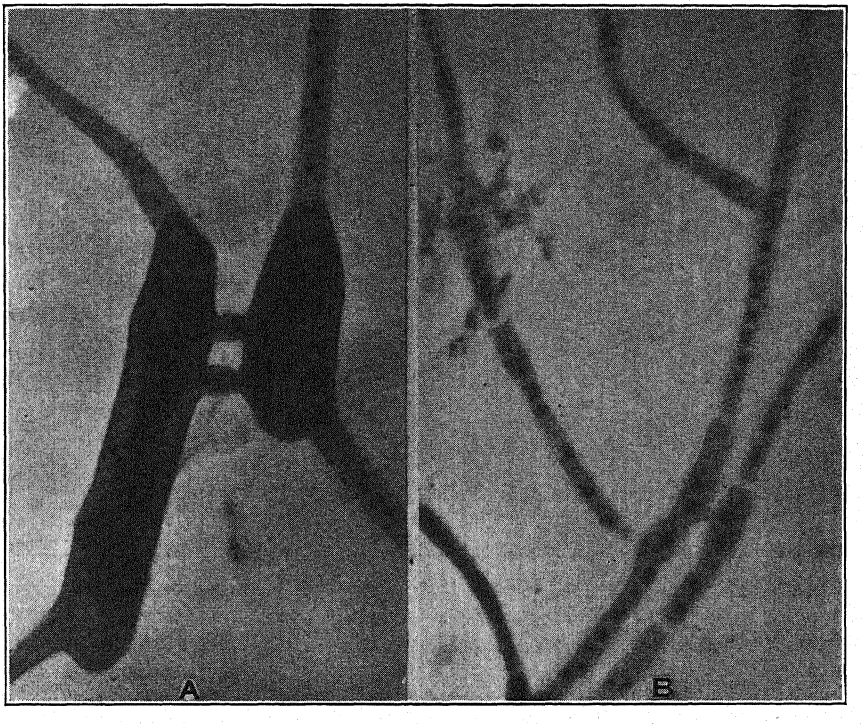


FIG. 1. A. Photomicrograph of two conidia of *Helminthosporium gramineum*. Two fusion tubes connect two different cells of each conidium. Nuclei are passing through the fusion tubes. The nuclei have been retouched slightly to bring them out more clearly. B. Photomicrograph of hyphae showing multinucleate cells. Fusion between two hyphae on the right.

Figure 2 shows quite clearly that the very young spore already contains several nuclei; hence it is quite possible that each may carry different factors for cultural characters and pathogenicity. The organism may then be truly heterocaryotic.

Not only are the cells of the conidia and mycelium multinucleate, but there may be interchange of nuclei between them. Conidia, germ tubes, and hyphal cells often fuse, as shown in figure 1, A and B. Evidently, nuclei pass through the fusion tubes from one cell to another, thus still further increasing the complexity of the nuclear complement of cells.

The evidence indicates strongly that heterocaryosis may account, at least in part, for variation in *Helminthosporium gramineum* and the production of new races. Even if all of the nuclei in the cells contained the same factors, which they probably do not, the differences in number easily could account for some differences in characters of different cells. The cells are multinucleate at the beginning, and nuclear interchange is common. It

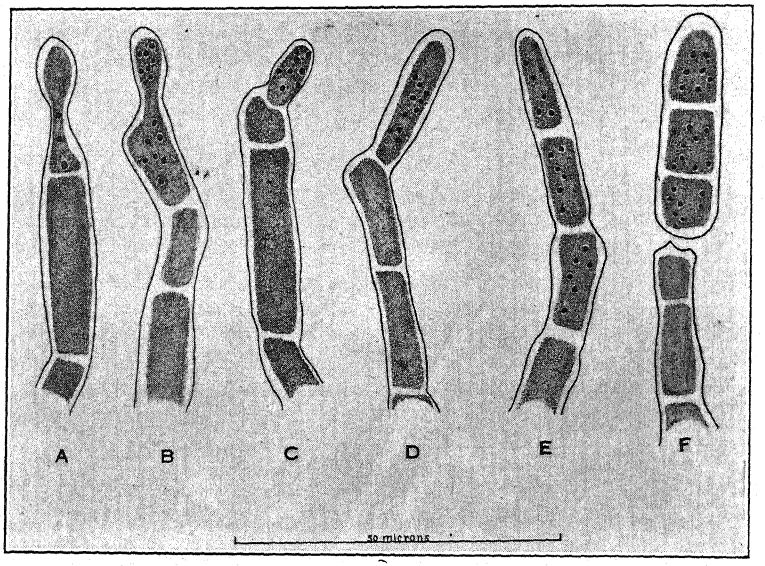


FIG. 2. The formation of conidia. Migration of several nuclei into the young spores shown at A, B, and C. Subsequent stages in the maturation of spores indicated at D, E, and F. Camera lucida drawings. Approx. ×1000.

seems reasonable, therefore, to suppose that the result may be the variation which is so characteristic of the organism.—T. W. GRAHAM, University Farm, St. Paul, Minn.

BOOK REVIEW

Goodey, T. *Plant Parasitic Nematodes.* xx + 306 pp. 136 figs. Methuen and Co., Ltd., London. 1933.

In this book is brought together for the first time the extensive and widely scattered information pertaining to the relationship between nematodes and plants. In the words of the author, the purpose of the book "has been to bring within the compass of a single volume the salient facts concerning all the known plant parasitic nematodes both as specific organisms and as producers of disease."

In chapter I are discussed the principal anatomical features of a typical nematode, methods for collecting and preparing for study specimens of plant-infesting species, methods commonly used for recording measurements and proportions, the present taxonomic status of the more important plant parasites, and morphological characters by which the genera Anguillulina, Heterodera, Aphelenchus and Aphelenchoides may be distinguished.

In the subsequent chapters, each of the more important parasites is discussed under the headings: historical, morphology, life-history, symptoms, pathology, hosts, geographical distribution and control, in so far as information on these phases is available. To this may be added a discussion of other facts peculiar to certain species. The question of biologic races as exhibited by *Anguillulina dipsaci* and *Heterodera schachtii* is discussed in the concluding chapter.

The author exhibits a thorough familiarity with the scattered and difficult literature on this subject: the material is well chosen and the subject matter well arranged. The press work is excellent with a commendable absence of typographical errors. The text is adequately illustrated with halftone and line cuts of a uniformly high grade. Each chapter is supplemented with a list of the more important literature references. The simple and readable style employed should make the book of value to the teacher, the general scientist, and the extension worker. The time consumed and labor expended in assembling information pertaining to plant parasitic nematodes have long been a distinct handicap to the specialists in this field. With a growing appreciation for the importance of diseases caused by these organisms, the need for such a text was becoming acute. It is fortunate that this book fulfills that need so well.—J. R. Christie.

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DISSEMINATION OF SOUTHERN CELERY-MOSAIC VIRUS ON VEGETABLE CROPS IN FLORIDA

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INTRODUCTION

The southern celery-mosaic virus (*Celery virus 1*) has been described by the writer (12). In field studies, reported in the present paper, of diseases caused by this virus, it was evident that a characteristic pattern of spread of the disease occurred in Golden Self Blanching and other varieties of celery, in Cocozelle and Golden Summer Crookneck varieties of squash, and in peppers of the California Wonder and World Beater varieties. There was some difference in disease spread in maize (sweet corn). The type of disease spread as it was noted in celery, peppers, and squash was unchanged in its attack on beds of 2 weed hosts, *Commelina nudiflora* L., and *Ambrosia elatior* (*artemisiifolia*) L.

In consulting the voluminous plant-virus literature one finds unanimity of opinion and numerous references to the fact that these diseases spread, but there has been but little detailed discussion of this phase of the problem. Allard (1) described how a small colony of aphids brought mosaic into a tobacco seed bed at the point of establishment of the colony. When transplanted from this seed bed sporadic cases of the disease occurred and, with increase in spread of insects, the disease was scattered throughout the field until nearly all plants were affected. Goss (8) stated that increase in aphid populations probably accounted for increase in potato-viroses. Gardner and Kendrick (7) found that tomato mosaic overwintered in wild hosts and could spread 40 rods. Doolittle and Walker (4) found that cucumber mosaic spread to cucumbers from diseased wild hosts in the field or along the edges. Folsom, Schultz, and Bonde (6) discovered in their work that dissemination of certain potato viroses extended across rows and from field to field. There was also a correlation between proximity and mosaic increase in the first rows next to diseased stock, and dispersal appeared to be accounted for by flight of infective insects. Smith (10) summarizes literature on virus dissemination and includes a list of citations (q.v.). It is notable that there have been no major detailed reports of field dissemination, pattern, or progress of spread.

MATERIALS AND METHODS

In the greenhouse studies the same strain of the virus was used as that employed in the writer's identification studies (12) of *Celery virus 1.* In all cases of apparent spread of the virus in the field, representative affected plants of different species from diseased fields were collected, the virus was recovered, and its identity determined by comparison with the known *Celery virus 1.* Aphids used in greenhouse studies were descendants of known cultures of *Aphis gossypii* Glov. maintained by S. P. Doolittle at Washington, D. C. Virus-free colonies were kept in insect-proof cages on healthy plants. Seeds of vegetable crops used in these studies were of named varieties obtained from reputable dealers, and specific identification of weeds were made by S. F. Blake of the Bureau of Plant Industry.

The field work reported in this paper was accomplished during the winters of 1930–31 to 1933–34, in Florida. General observations were made repeatedly in several parts of the State, and careful counts, maps, and detailed studies were made in the district around Sanford, Fla.

PATTERN OF DISTRIBUTION OF DISEASED PLANTS IN THE FIELD[1]

Diseases caused by the southern celery-mosaic virus spread in a generally characteristic pattern in fields of various varieties of squash, pepper, and celery. Initial appearance usually occurs on the edge of the field where there may be an infection source. This may be diseased crop plants of the same species or of other species or it may be a fringe of diseased wild host plants. After introduction into the field the disease spreads inward but it does not necessarily extend to make a fan shaped pattern, nor is there a frontier border of spread progressing in an unbroken line as a wave would advance. Dissemination is evident principally as a successive development of small scattered areas which, upon expansion, coalesce and in turn result in further infection and widely dispersed centers of disease. This may continue until the whole field is completely involved.

Spread of the celery virosis on celery, *Apium graveolens* L., was studied with particular care during 4 winters in sections of a number of fields in the Sanford district. One such plot (Fig. 1), studied in the winter of 1932–33, is a fairly typical example of the spread of this disease. This plot was planted in November with 1050 celery plants from a well sprayed virosis-free seed bed. In this plot there were 14 rows, 30 inches apart, with plants, all of the Golden Self Blanching variety, 3½ inches apart in the row. Two small patches of weeds, composed largely of *Commelina nudiflora* and *Geranium carolinianum* L. were located along the eastern edge of the plot. Both species were diseased by a virus that proved to be *Celery*

[1] The diagrams in this section were prepared by F. S. Beecher, Bureau of Plant Industry, from the writer's field diagrams.

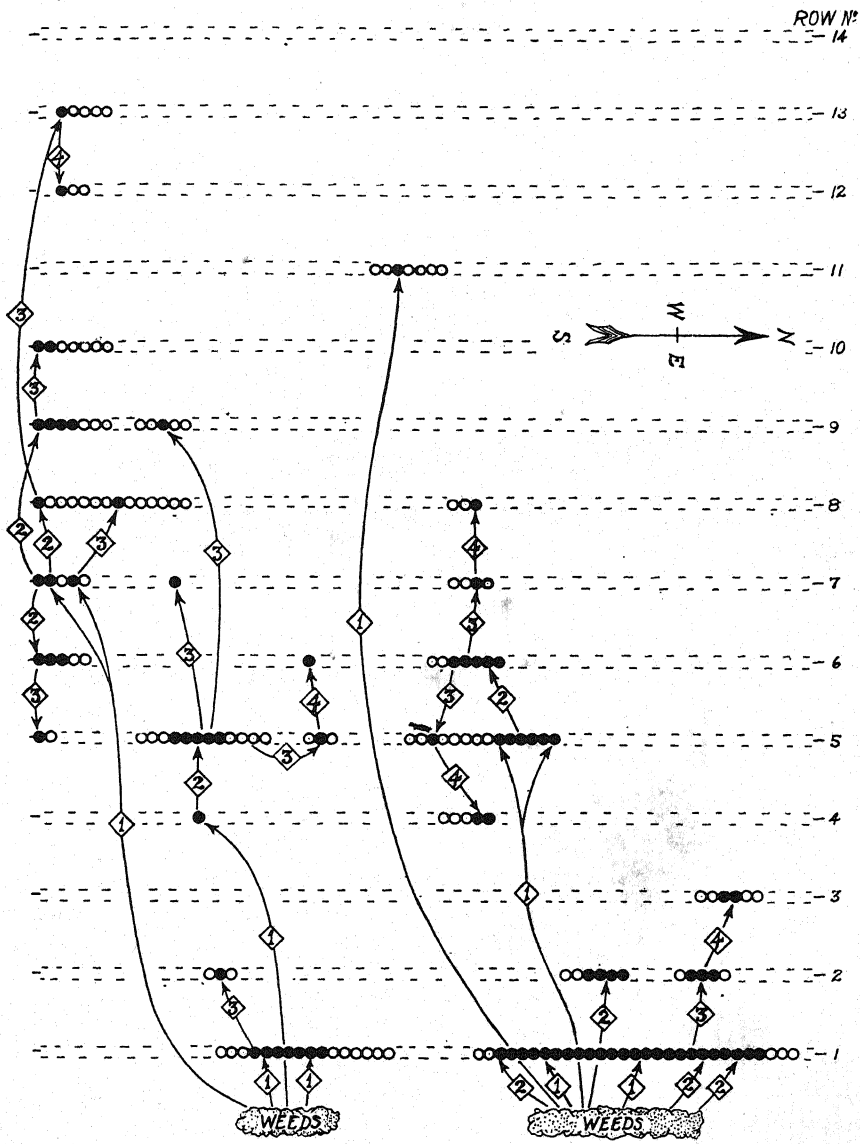


FIG. 1. Diagrammatic representation of results from field studies of spread of celery virus in Golden Self Blanching celery plants. Primary diseased plants are represented by black spots, circles are secondary diseased plants adjacent to primary infected individuals, and arrows represent probable course of travel of viruliferous aphids to new areas of infection. Arrows numbered 1 lead from weeds acting as original sources of infection to areas of plants that first appeared as diseased on December 1. Arrows numbered 2 lead from old to new areas of infection noted December 11. Aphids disappeared from weeds at this time but multiplied rapidly on celery. Arrows numbered 3 lead from previously infected areas to plants found diseased on December 24. Arrows numbered 4 represent course of spread to new diseased areas observed January 12. On January 20 there were few aphids and no new diseased areas. Newly infected plants all occurred in rows next to plants already diseased. Generally speaking, spread of the disease was in all directions, though greatest length of spread was from east to west, the direction of the warm east trade winds prevalent during the period of these observations. (For successive increases in numbers of diseased plants, see text and the figures given for plot I in table 1.)

virus 1, and were liberally infested with *Aphis gossypii,* which were multiplying upon them at the time the plot was planted. These aphids left the weed hosts and moved to the celery shortly after it was put into the field. This migration is common and is not necessarily induced by planting and cultivation of fields adjacent to weed patches. Wingless aphids were found to crawl away from plants colonized by aphids, at almost any time and in any direction, in search of new pasturage. Winged individuals were observed to leave their parent colonies early in the morning. They flew toward the sun but were usually carried back on the warm and light easterly breezes then prevalent, alighting at considerable distances west of the plants from which they originally flew.

On November 29 the plot had been planted 15 days. It was carefully examined and some plants with suspicious markings were noted but none could be found with definite symptoms of virus infection. Diseased weeds on the edge of the field had several aphids on them, and 40 celery plants in the field were found infested with 1 to 7 winged and wingless aphids. On December 1, aphids were found in about the same numbers on celery and weeds, and there were 6 areas in the field in which a total of 26 virus-diseased celery plants were found. These plants all seemed to have been originally inoculated by viruliferous aphids coming from the weed patches, as indicated on the diagram (Fig. 1) by arrows numbered 1. One hundred ninety-two plants carried aphids at this time, and it was evident, since only 26 of these were diseased, that not every plant that harbored an aphid had succumbed to virus infection.

On December 11, no aphids were found on the weeds at the edge of the plot. Aphids were, however, becoming more numerous on the originally infested celery plants, were spreading to surrounding individuals. Six new infection areas were noted (Fig. 1) that resulted apparently from aphid migration both from weed patches and from the first areas of plants infected. Thirty-seven new diseased celery plants, making a total of 62 plants with mosaic symptoms, were recorded, the apparent source of new infections traced and indicated by arrows marked 2. Aphids were not abundant anywhere in the plot on December 11. By December 24, further multiplication and spread of viruliferous aphids had occurred. No aphids could be found on the weeds, but 11 new virus-infected areas of celery plants had appeared with 12 newly infected plants, all of which (Fig. 1) seemed to have been initiated by aphids from originally infected plants noted December 11. The course of this spread was designated by arrows marked 3 (Fig. 1). On December 24 aphids had become more plentiful in this field, and the first secondary spread of the disease in plants next to the primary infected individuals in the row was definitely observed. Twenty such individuals were found and are denoted as circles in the diagram (Fig. 1).

The plot was not visited again for over 2 weeks and on January 12 further spread had taken place and the course of spread into the 7 new areas (Fig. 1) was traced. This is signified by arrows marked 4 on the diagram. It appears that in every case this extension originated in primary infected celery plants that had been previously diseased. A marked diminution in aphid population also was noted. At the same time, 33 celery plants, part of those designated by circles in figure 1, had succumbed to the disease and evidently had been inoculated by insects from diseased plants growing next to them in the same row. The next observations were made January 20, when aphids had all but disappeared and no new areas of infection appeared in the field. There were, however, 37 additional plants infected through contracting the malady from other individuals next to them in the same row. At that time a total of 175 plants were diseased.

It was not possible, because of pressure of other field work, to make regular subsequent counts of diseased plants. General observations, however, were made. Very few aphids could be found and almost no increase in disease occurred until late February, when a severe infestation of aphids came in from infested, though virus-free, squash in a near-by field. This adjoining field of squash had recently been chopped down preparatory to replanting. Upon harvesting the plot of celery, March 24, the weeds were found to be free from aphids, though large numbers of the insects were present on the celery. There were 928 diseased plants in the plot, which was 88 per cent of the population. A week after harvest the weeds at the edge of the plot were again found to be well infested with numbers of *Aphis gossypii.*

A more general view of the pattern and course of spread of the celery virus in a celery field was obtained in the winter of 1930–31. Along one edge of this field, diagramatically represented in figure 2, A, B, C, and D, there were 7 patches of weeds all having severe symptoms of virus attack. The weed species consisted of *Commelina nudiflora, Geranium carolinianum, Ambrosia elatior* (*artemisiifolia*), *Bidens leucantha* (L.) Willd., and *Chenopodium botrys* L. Subsequently, by inoculation studies, all these weeds were proved to be infected with *Celery virus 1* with the exception of Bidens and Chenopodium.

The disease appeared in the field first along with a scattering of aphids, *Aphis gossypii,* in a few plants close to virus-infected weed patches. By December 24, a total of 16 diseased plants were located and the areas in which they were found were noted (Fig. 2, A). The second date of observing virus-disease progress in this field was January 20, 27 days later than the first (Fig. 2, B). There were 110 diseased plants and the spread extended westward and was distinctly correlated with the location of beds of diseased weeds. Rapid and concentrated invasion was particularly notable

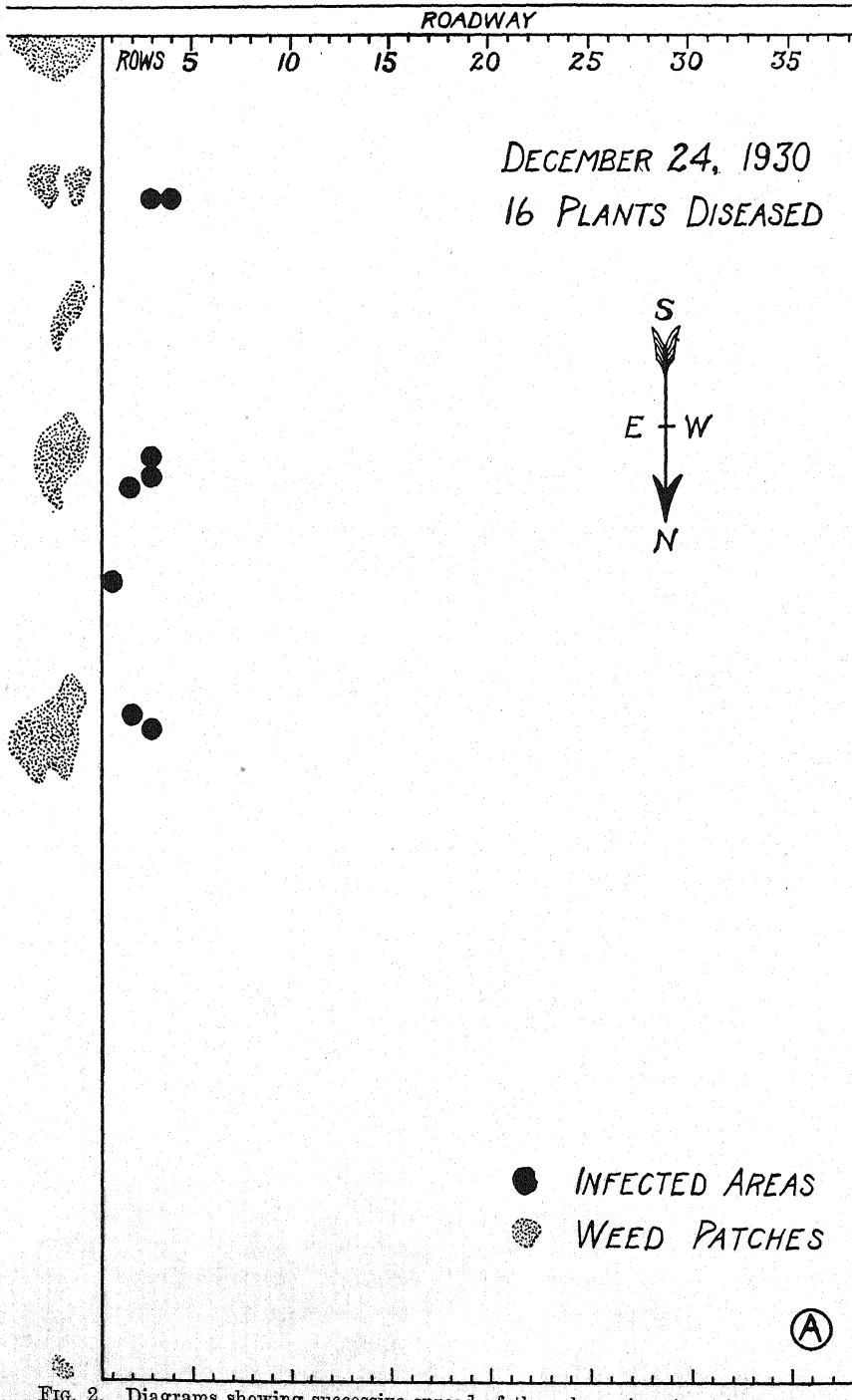
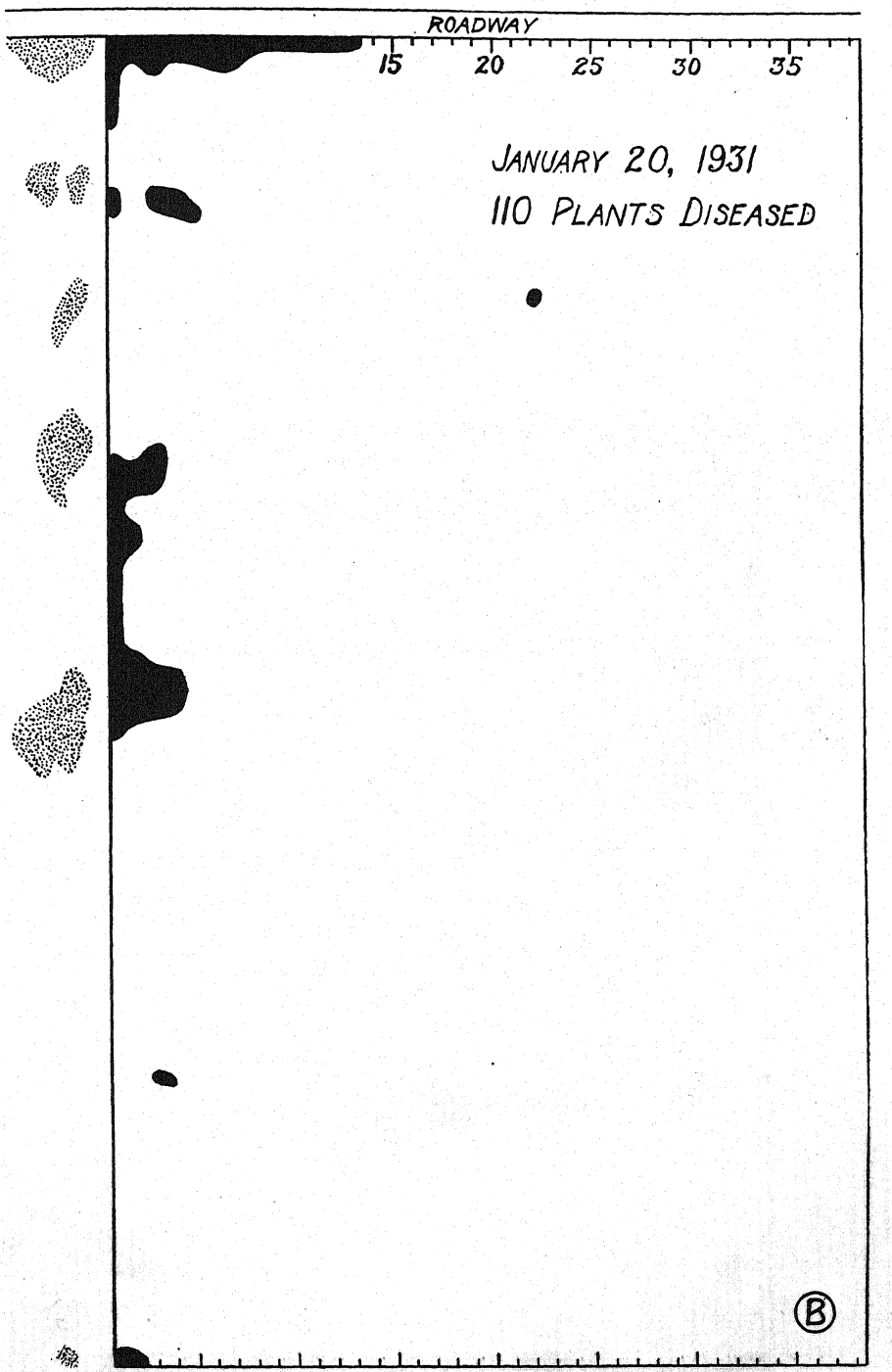
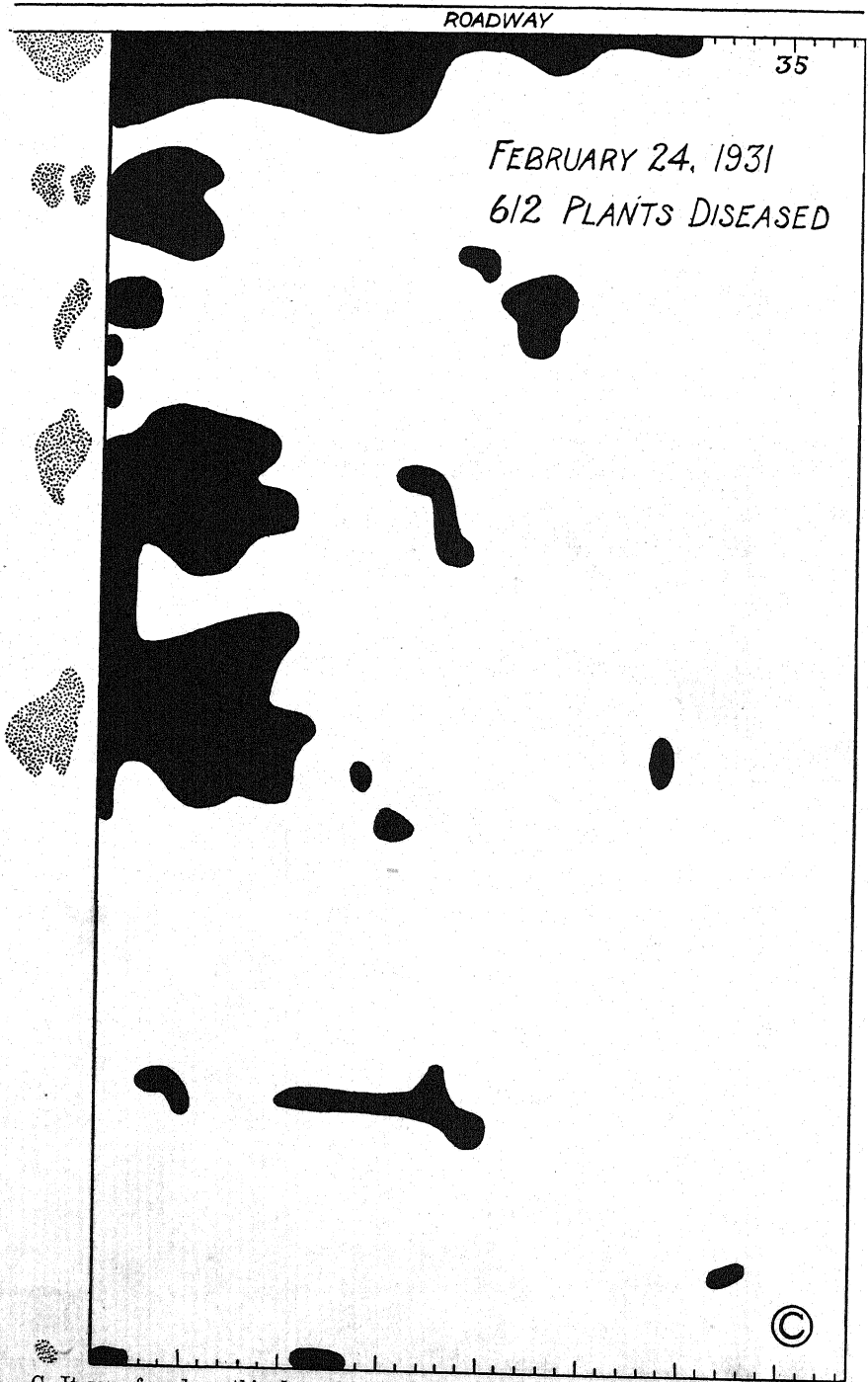


FIG. 2. Diagrams showing successive spread of the celery virus in a field of Golden Self Blanching celery, near Sanford, Florida.

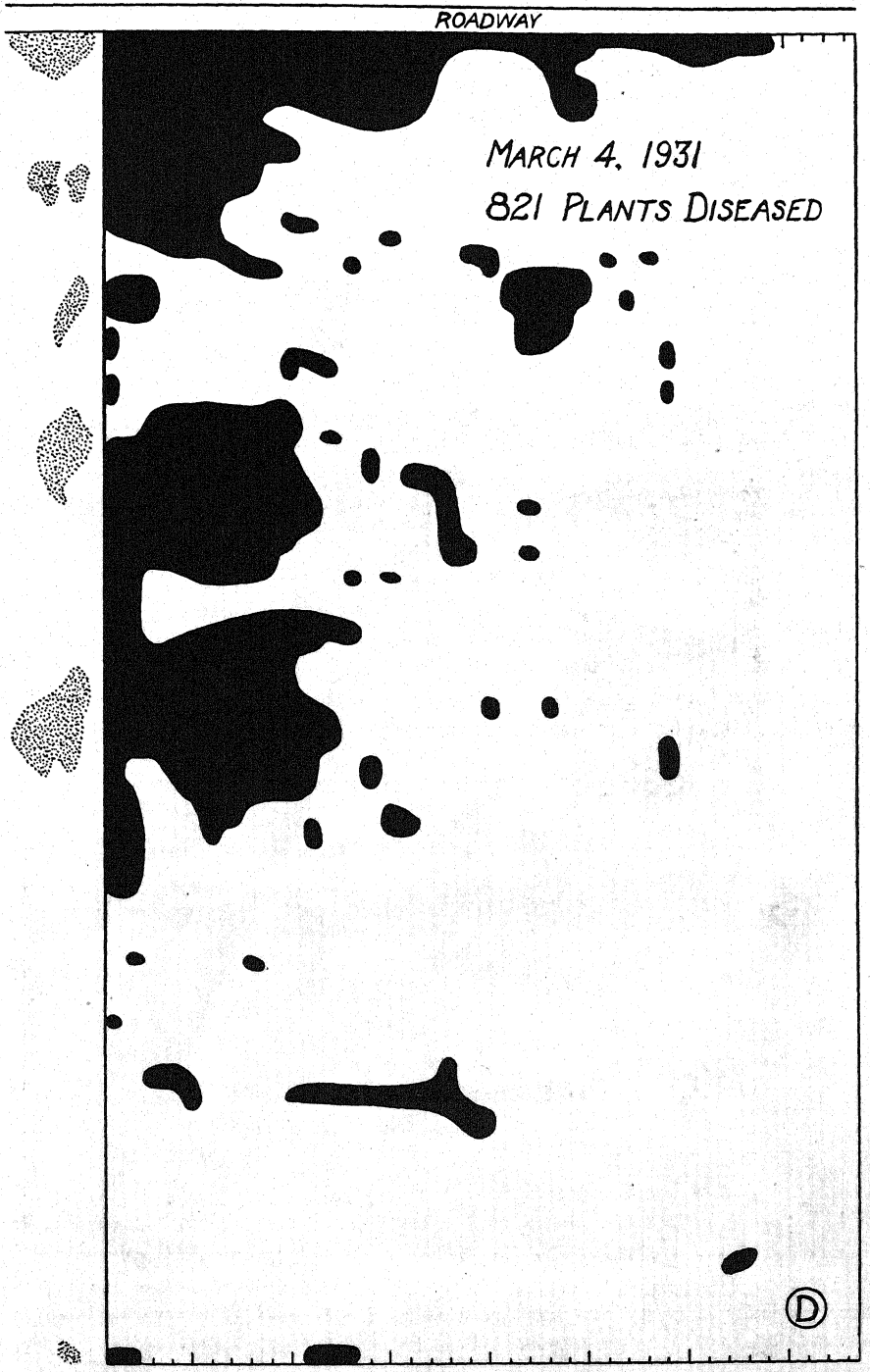
A. Note that all diseased plants to be found at the beginning of observations were in the first 4 rows, and were definitely correlated with position of weed patches at eastern edge of field.



B. Spread had progressed rather slowly in a month's time but considerable increase in numbers of diseased individuals was, nevertheless, noticeable. There were also 2 well-isolated areas where disease appeared, one of them being about 45 feet from the nearest infected plants.



C. It was found on this date that spread had progressed very rapidly in the ends of the rows along the weed-free roadway. Also, that general expansion of infected areas occurred at the time of appearance of numerous isolated areas of infection.



D. The last observations were made somewhat over a week later. The total number of diseased plants had increased greatly, as well as the numbers of isolated areas of disease.

along the weed-free roadway on one side of the field. The ends of the first 13 rows of plants abutting upon this roadway were especially affected. The third observations were made 35 days later, on February 24. At this time, 612 plants showed virus attack (Fig. 2, C), spread had advanced further into the field, and at considerable distances there were scattered local areas of infection completely surrounded by healthy plants. The disease appeared to be spreading more rapidly in a westward than in an eastward direction. The last counts and observations were made on March 4 (see Fig. 2, D). In a period of 8 days the total of diseased plants had increased to 821, the general regions of infection had become considerably extended, and numerous small assemblages of mosaic plants had become established around the outside edges of the areas of spread, though the smallest infected areas are not indicated in figure 2, D. This celery was harvested nearly 4 weeks after the writer made his last counts. The owner of this field reported that the disease had spread in severe form during the remainder of the month of March, to considerably more than twice the area affected on March 4, and that all sections of the plot were supplied with numbers of small areas of diseased plants. Severe losses are sustained in such a field at harvest time, as is illustrated in figure 3.

In studying the pattern of spread of celery virus in squash and pepper fields it was found to be of the same type as that described for the trouble in celery. It differed especially in squash fields in that infected areas were

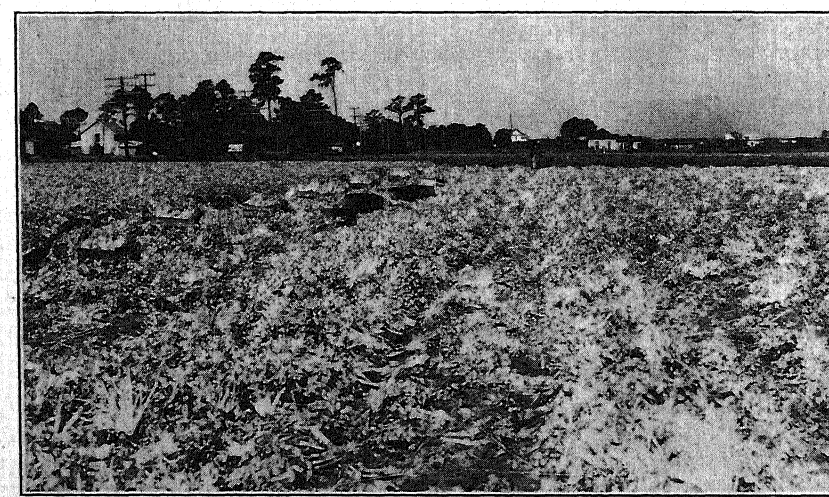


FIG. 3. Photograph toward center of a celery field at harvest time showing the large majority of celery plants that were infected with southern celery mosaic so disfigured that they were cut down and left in the field because of unsuitability for shipment. (Photograph by S. P. Doolittle.)

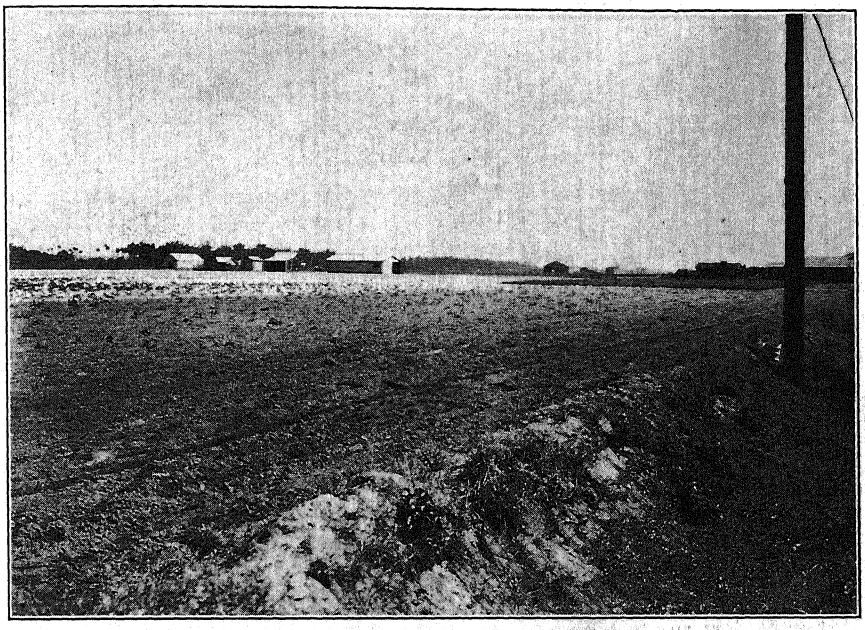


FIG. 4. Photograph of month-old 10-acre field of Golden Summer Crookneck squash severely diseased with *Celery virus 1* originating from infected weeds (*Commelina nudiflora, Ambrosia elatior,* and *Geranium carolinianum*) growing in the shallow ditch seen in the foreground. This field had a perfect stand of squash seedlings at the time the virus started to spread. Aphids migrated from the weeds, infected the squash plants, multiplied, and migrated rapidly to all parts of the field. Note especially dead and dying plants in the bare area over an extensive portion of the field, with a few rows of more mildly affected squash plants in the distant background. Field corn was being planted between the squash rows at the time this picture was taken, and sometime later the squash plants were all hoed out, no salable crop having developed.

more conspicuous and more rapidly involved (Fig. 4). In fields of the sweet varieties of maize, spread was more or less confined to plants near the field edge, where the original source of infection occurred. Isolated diseased areas were found occurring well into the field, but they did not serve as infection centers from which the disease spread greatly, as in fields of celery, squash, and peppers. The accompanying illustration (Fig. 5) shows a maize field in which the disease is mostly confined to the first row next to celery-virus-diseased weeds, Ambrosia and Commelina, in a roadside ditch. In this field nearly every plant in the first row was infected and the disease was prevalent in the first 5 rows. While infected plants were found 25 rows away from this edge, the majority of the infected plants occurred close to the field edges.

In studying the spread of the virus from plant to plant in growths of the weed, *Commelina nudiflora,* the pattern of spread appeared similar to the typical form described as occurring in celery fields. In January, 1933,

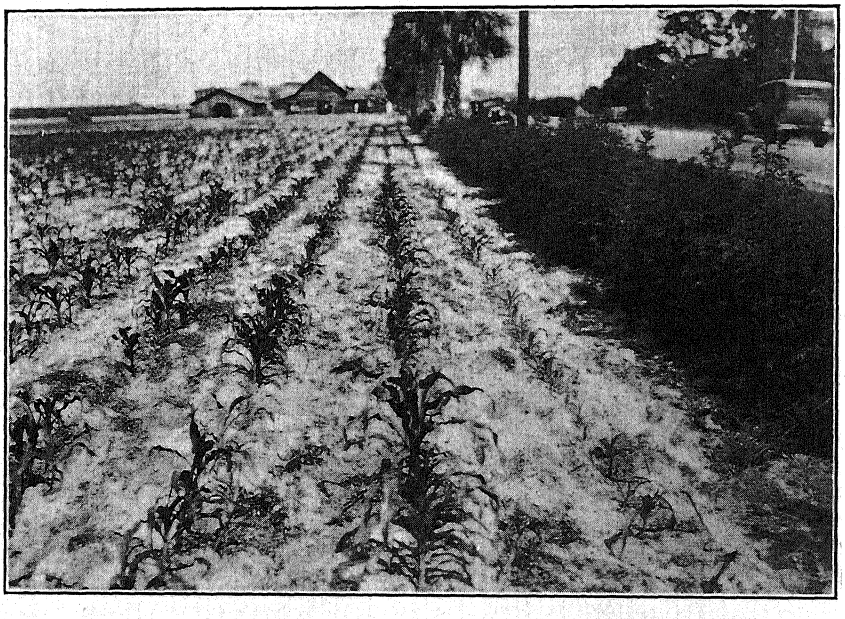


FIG. 5. The spread of *Celery virus 1* in a field of maize (sweet corn) by aphids (*Aphis gossypii*) that had fed on infected weeds (*Ambrosia elatior* and *Commelina nudiflora*) growing at the edge of the field. Note stunting of diseased corn plants in first row. Nearly every plant in this row was affected, the disease was most prevalent in the first 5 rows, but could be found with ease 25 rows from the edge.

a 4-acre field, which had been uncultivated for a season, was found covered under the sparse growth of a leguminous cover crop, by an almost complete mat of *C. nudiflora*. This mat, apparently the result of germinated seeds, was free from virus infection with the exception of a few small diseased areas occurring toward the middle of the field, and a fringe of infected plants along the edge where there were some diseased *Geranium carolinianum* and the remains of an old field of celery-virus-infected peppers. In a month's time the fringe of infection had advanced into the field and become amalgamated with the isolated spots. The front line of infection had extended about 30 feet in from the edge of the field where the infection originated. Spread of the disease had almost ceased by the last of March, though it proceeded during the summer, and by the first week of September nearly all Commelina plants in the field were diseased. Several abandoned fields where the ragweed, *Ambrosia elatior*, had been allowed to grow were watched for spread of the disease. The pattern of spread of the celery virosis in these patches of weeds was essentially the same as that described for celery.

INCREASE IN NUMBERS OF DISEASED PLANTS IN CELERY FIELDS

Observations were undertaken in celery fields to study increase of disease in plots and the number of diseased plants in isolated areas where infection originated from single diseased individuals.

Successive counts of virus-diseased plants were secured, and the data have been assembled in tables 1, 2, and 3. Close study has shown that consecutive increases in the number of infected plants in fields are dependent on: the sources of infection, proximity of the sources to the crop under investigation, temperature and humidity relations, multiplication and migratory movements of the insect vector, size and shape of the plots, and methods of culture employed in the crops affected. Work on these factors is not complete and no attempt has been made here to correlate the data with increases of the disease.

TABLE 1.—*Increases in numbers of celery-virus-diseased plants at intervals during one season in plots of Golden Self Blanching celery*

Plot	Plants in plot	Observation	Plants diseased	Origin of virus	Presence of aphids
A	41,838	Dec. 24, 1930	16	Weeds	Weeds and celery
		Jan. 20, 1931	110		Celery only
		Feb. 24, "	612		" "
		Mar. 4, "	821		" "
I	1,050	Nov. 29, 1932	?	Weeds	Weeds and celery
		Dec. 1, "	27		Celery only
		" 11, "	62		" "
		" 24, "	94		" "
		Jan. 12, 1933	134		" "
		" 20, "	175		" "
		Mar. 24, "	928		" "
L	1,000	Nov. 6, 1933	1	Weeds and celery	Celery
		" 11, "	5		"
		" 21, "	15		"
		Dec. 1, "	41		"
		" 12, "	88		"
		Jan. 1, 1934	169		"
		" 12, "	288		"
		" 24, "	380		"
		Feb. 17, "	552		"
		" 27, "	736 (at harvest)		"
		Mar. 6, "	.....		"
N	1,000	Nov. 6, 1933	1	Celery	None
		" 11, "	1		"
		" 21, "	1		"
		Dec. 1, "	1		"
		" 12, "	1		"
		Jan. 1, 1934	2		Celery
		" 12, "	6		"
		" 24, "	16		"
		Feb. 17, "	37		"
		" 27, "	42 (at harvest)		"
		Mar. 6, "	.....		Weeds

The data presented in table 1, indicate the successive increases of diseased plants that occurred in plots. The mere occurrence of diseased plants in fields does not imply disease spread. Diseased plants may long be present in fields before spread of infection begins. The writer has known many instances of field infection derived from isolated diseased plants obtained from seed beds containing a few infected seedlings at planting time. In 4 such fields aphids had been destroyed in the seed bed and did not become abundant later in the season, diseased plants escaped infestation, and the virus was not spread to other plants during the whole course of the season. In plot N (Table 1) a single diseased plant was located in the center of a 1000-plant plot, and did not become a source of disease spread for 2 months, when aphids had colonized on it and then proceeded to spread the virus. In plots A, I, and L, in which spread occurred from the first, infection originated from weeds at the edge of the field and aphids were always present.

In the 1930–31 season, which was unfavorable to the spread of southern celery mosaic, observations were made with regard to spread of disease around 20 single newly infected individuals in marked locations in the fields (Table 2). These studies were made on 2 adjacent farms, and the isolated

TABLE 2.—*Spread of southern celery-mosaic virus to other individuals about single, originally diseased Golden Self Blanching celery plants in the field (Winter 1930–31)*

Original plant	Date diseased	Spread in original row		Spread to adjacent rows[b]		Spread at close of season	
		No. plants	Farthest spread[a]	No. plants	Occurrence	Total plants	Days required
a	Dec. 9	2	4 inches	0	.....	2	87
b	" 11	5	12 "	0	.....	5	85
c	" 11	13	36 "	32	both sides	45	84
d	" 11	5	12 "	3	one side	8	85
e	" 20	4	12 "	5	both sides	9	76
f	" 20	13	28 "	39	" "	52	74
g	Jan. 1	6	20 "	1	one side	7	65
h	" 1	2	8 "	0	.....	2	63
i	" 1	4	8 "	3	one side	7	65
j	" 1	5	8 "	4	both sides	9	64
k	Dec. 24	4	16 "	0	.....	4	81
l	" 24	7	16 "	10	one side	17	80
m	" 24	5	8 "	0	.....	5	79
n	Jan. 1	2	4 "	1	one side	3	64
o	" 1	4	8 "	2	" "	6	63
p	" 1	5	12 "	12	both sides	17	65
q	" 1	2	4 "	5	" "	7	65
r	" 1	14	52 "	27	" "	41	65
s	" 1	3	8 "	9	" "	12	65
t	Dec. 7	5	16 "	1	one side	6	89

[a] Plants were set 4 inches apart in the row.

[b] Rows were 30 inches apart in the field.

locations chosen were widely scattered, between 75 and 150 feet away from patches of virus-infected weeds or field edges. It is of considerable interest that so much variability occurred in the amount of spread of mosaic from an originally diseased plant to those surrounding it. In 2 cases (Table 2, plants a and h) the disease spread to only one other plant next a diseased individual, while within a few rods of those plants the disease spread to 51 plants from a single primary infected individual (Table 2, plant f). Of equal interest is the fact that in 1930–31, a season in which there was but little celery mosaic spread, as many as 41, 45, and 52 plants became diseased in areas in the field all started from single infected individuals. Similar studies were again attempted in 1931–32 and 1932–33. These seasons were warm, and in all cases data were so complicated with vigorous scattering spread of the disease that counts were not completed. In these seasons the disease spread from individual plants would have been considerably greater than any reported in table 2.

One method of expressing the importance of a disease in the development of the host is to determine the total number of plants in a certain population affected with the disease. In studying the virus of southern celery mosaic, counts were made in field plots at the time of year when the disease was first making its appearance. Later on, another count was made to determine in some measure how much the disease had increased. This increase of diseased plants (Table 3) was obtained in 14 different plots of celery during 4 seasons of observations. In addition, general con-

TABLE 3.—*Numbers of plants infected with Celery virus 1 at beginning and close of season of observation on field plots of Golden Self Blanching celery*

Plot	Total plants in plot	Counts of diseased plants		Date of first count	Days to last count
		First count	Last count		
A	41,838	16	821	12/24/30	67
B	39,600	66	34,542	12/11/30	83
C	29,896	36	3,348	12/11/30	86
D	4,200	14	43	12/24/31	22
E	4,200	67	308	12/24/31	22
F	5,418	126	1,592	12/1/31	44
G	5,160	138	1,508	12/24/31	22
H	14,133	8	89	12/24/31	22
I	13,608	7	76	12/1/32	46
J	5,418	92	1,572	12/1/32	46
K	1,050	26	928	12/1/32	115
L	1,000	1	736	12/21/33	113
M	1,000	1	6	11/21/33	83
N	1,000	1	42	12/12/33	113

firmatory observations were made on a large number of fields in the neighborhood of the plots. It is not exceptional for such large proportions of plants to become diseased as are, for example, indicated in plots B, C, F, G, J, and K, and this furnishes further verification of the fact that the virus, unless curbed in its attacks, might well become a limiting factor in the crops produced from certain cultivated host plants. It should be noted in passing that the virus has a very wide host range, subsequently to be reported upon, and that it is especially severe in plantings of pepper and squash.

FACTORS INFLUENCING FIELD DISSEMINATION

The primary occurrence of the celery virus in cultivated crops depends on the presence of adjacent diseased plants affected with the celery virus, and on the agency of spread. Methods of spread without aphids have been studied in the field, and experimentally under insect-free conditions. In a minor way spread of the virus results, in pepper and squash fields, from bruising incurred in the handling of plants in the hoeing process, and in the harvesting of fruits, while in celery it may take place by the continued bruising and exceedingly close contact of these plants as they stand in the row after they are about half-grown. Spread from contacts of celery plants in the row, however, may be practically disregarded as a factor of much economic importance in the dissemination of celery virosis.

The work of Doolittle and Wellman (5) proved that *Celery virus 1* could be transmitted by *Aphis gossypii* from *Commelina nudiflora* to celery, and *vice versa,* that the aphid might also transfer the virus from *Physalis lagascae* R. & S. to celery, and that the insect could successfully inoculate cucumber, tomato, *Physalis pubescens* L., and tobacco. The writer has recently reported (13) that *Aphis gossypii* carried the celery virus to certain of the Gramineae: *Zea mays* L., *Euchlaena mexicana* Schrad., *Holcus sorghum* L., and *Secale cereale* L. The writer also reported (14) that *Aphis gossypii* and *A. maidis* Fitch carried the virus to 2 species of banana, *Musa cavendishii* Lamb and *M. sapientum* L. Other aphid species are being studied as vectors of *Celery virus 1.*

The writer has pointed out (11) that *Aphis gossypii* is the important insect vector of the celery virus on celery around Sanford, Florida, and in that district it is called the celery aphid. The question arose as to whether *A. gossypii* might also act as the vector of *Celery virus 1* in a number of plant species not heretofore studied under controlled conditions but to which the virus was known to spread in the field. From 50 to 80 plants of every variety and species used, making up 5 to 8 series in each case, were studied in the greenhouse. It was found that *A. gossypii* was a potent vector of celery virus in the following varieties and species of plants: *Cucurbita pepo* L. var. *condensa* (Cocozelle and Golden Summer Crookneck

squashes), *Capsicum annuum* L. var. *grossum* (California Wonder and World Beater peppers), *Ambrosia elatior,* and *Geranium carolinianum.* Photographs of pepper and squash plants diseased with the celery virus upon inoculation with viruliferous *Aphis gossypii* in the greenhouse are presented in figure 6.



FIG. 6. Plants of (A) Cocozelle summer squash and (B) California Wonder pepper, disease-free and infected with the celery virus. These were inoculated by the use of viruliferous *Aphis gossypii.* In both cases the diseased plant is the one that appeared characteristically stunted and did not produce usable fruits of any sort. The healthy plants continued to grow vigorously and good fruits were harvested from them.

The proximity of occurrence and presence of the first diseased plants in fields in relation to original source of infection, were found to be almost perfectly correlated with distance and direction of travel by aphids walking over the ground away from infected border plants (Figs. 1, 2, 3, and 4). Wingless aphids have been observed many times, both in the field and in the greenhouse under cheesecloth cages, walking away from diseased plants for considerable distances and ascending healthy individuals upon which colonies became established and virus infection appeared. Winged forms of *Aphis gossypii* occur commonly in Florida but have never been found by the writer in great abundance around Sanford. The few times that flight has been studied were early in the morning, and the aphids in general flew towards the sun, though they were easily picked up by the easterly breezes and carried westward 25 to 150 feet from the plant from which they originally took flight. Moreover, the occurrence of virosis in widely scattered areas (Fig. 2, C and D), from which the trouble subsequently spread locally, has been found to occur at distances and in directions from original sources of infection that correlate well with the observed flights of winged aphids.

In the fields of plants studied, the celery virus spread most rapidly and extensively in Cocozelle and Golden Summer Crookneck squashes. In these

the plants averaged a little more than 9,500 to the acre. The rows were 36 inches apart with plants at about 18-inch intervals in the rows. Aphids multiplied very rapidly on these plants, soon became crowded, and travelled with readiness, carrying the virus across the field. The virosis also spread readily over pepper fields. These fields contained rows 30 inches apart with plants about 12 inches apart in the row, and approximately 17,000 plants per acre. Although this plant apparently is not so favorable a host for *A. gossypii* as is squash or celery, the aphids multiplied with ease and carried the celery virus from one part of the field to another. In celery there were around 60,000 plants per acre. The rows were 30 inches apart and the plants so close together (about 3 inches in many cases) that they soon made a solid and compact hedge of tender foliage in the row. Compactness of growth in celery was, perhaps, somewhat of a deterrent to spread of the celery virus, since aphids need go only a short distance to find an ample supply of leafy, vigorous, and succulent growth upon which to feed and multiply. In the cases of plants that have more space between them in the field, travelling aphids have to go farther, and have greater chance of passing between the plants as they go from one portion of the field to another. The spread of celery virus in maize (sweet corn) seemed to stop after the plants were about a foot high, when the aphids appeared to discontinue their feeding and went to other crops and bordering weeds.

THE NATURAL HISTORY OF *Celery Virus 1*

The celery virus is apparently endemic to the Florida peninsula. The writer found, with significant consistency, plants of *Commelina nudiflora* and *C. communis* L., infected with celery virus, growing wild, in many locations along river edges, in regions of the Everglades, and on the outskirts of hammocks miles away from habitations where it is impossible to conceive that, in every case, the plants were escapes from cultivation. In a recent trip to Cuba celery, squash, and pepper plants that appeared to be affected with typical virosis of celery, were found closely associated with virus-infected Commelina. Cook (2), in Puerto Rico, described and illustrated *C. longicaulis* Jacq. showing symptoms of what appeared to be typical celery virus. Kunkel (9) also has reported that, in Hawaii, *C. nudiflora* showed symptoms of virus infection.

Celery virus 1 occurs on several weeds in Florida, though in that region it has as its most important weed host *Commelina nudiflora* (*cf.* Doolittle (3) and Doolittle and Wellman (5)), as was proved by the writer through eradication of this weed alone (11), by which method it was possible to cut down spread of this virus to celery fields to a very marked extent. The virus exists in warm countries from season to season, over trying periods of extreme drought or rain, in the stems and buds of *C. nudiflora.* This

seed plant has no distinct season of quiescence in the form of seed or overwintering root stalk, but vegetates continuously and thus keeps the virus alive, and, in Florida, is not readily killed in nature by either excessively dry or wet weather.

Aphids act as vectors of the virus, carrying it from infected perennial plants of little or no apparent economic importance to those of annual type, where it multiplies and spreads vigorously. When the annual plants die or are chopped down, aphids leave and carry the virus back to nearby perennials, reestablishing themselves on old plants, or colonizing and infecting the young perennials, which act as reservoirs of the virus over periods unsuited to growth of the more tender plant species. It is upon the advent of new growth of many of the annuals that dissemination of the disease becomes most notable. The virus-carrying aphids migrate back from the less succulent and palatable reservoir hosts to annual crops and weeds, and the virus then becomes economically significant.

SUMMARY

The southern celery-mosaic virus (*Celery virus 1*) causes diseases that were found to spread in a generally characteristic manner in fields of various varieties of squash, pepper, and celery, and in the weeds *Commelina nudiflora* and *Ambrosia elatior*. It differs somewhat in its spread in maize.

The generally characteristic pattern of spread was studied in detail on celery. Initial appearance usually occurs on the edge of the field where there may be an infection source. This may be diseased weeds or other crop plants. The disease spreads inward through the successive development of small scattered areas that coalesce and, in turn, result in further infection and widely dispersed centers of disease. This may continue until the whole field is involved. In sweet corn the majority of infections occur in young seedlings toward the edges of the field next to infection sources. The disease spreads only while the corn is in the seedling stage.

There is some spread of this virus through mechanical means, but large increases in numbers of diseased plants in celery were observed to be dependent upon the occurrence of large numbers of the aphid vector, *Aphis gossypii*. Such a large proportion of plants becomes diseased that the virus, unless checked in its attacks, might well become a limiting factor in the production of certain cultivated host plants.

Observations showed that field dissemination depends largely on the movement of viruliferous aphids either in walking from plant to plant or in flying from one part of the field to another. The most rapid and extensive virus-disease spread occurred in squash fields and to a less extent in peppers. These plants are planted some distance apart in the field. In

fields of celery, which contain a great many more plants per acre, the disease was somewhat deterred in its spread by the excesssive amount of foliage encountered by the aphids.

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RELATION OF FUSARIUM AND HELMINTHOSPORIUM IN BARLEY SEED TO SEEDLING BLIGHT AND YIELD[1]

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INTRODUCTION

Barley seed blight is a disease complex that has received relatively little attention except when barley goes into market channels for malting or feeding purposes. As much of the barley produced in the Northwest is fed on the farm, seed blight often is overlooked. Recently, however, because of several outbreaks of scab, caused by *Gibberella saubinetii* (Mont.) Sacc., greater attention has been paid to the so-called blight of barley seed. Furthermore, the revived demand for good malting barley also has stimulated interest in the problem. The feeding value of barley would of course be considerably reduced if a large percentage of the blighted kernels were affected with *G. saubinetii,* which is likely to be toxic to pigs. The general impression that blighted barley is not likely to be very desirable for malting purposes also has intensified the problem. Under the circumstances there naturally is discrimination against blighted barley offered on the market for feeding or for malting purposes. There is another very important aspect to the problem from the farmer's standpoint. What is the value of blighted barley for seed? And can it be improved by seed treatment?

In Europe, seed disinfection is practiced extensively for the control of root rot of cereals, but in the United States very little has been done along this line. There are, however, rather conflicting reports on the effect of various fungicides on seed germination, vigor of growth, and yield. Therefore, in 1932 a study was begun on the relation of the microflora of barley seeds to germination, stand, vigor of growth, seedling blight, and the value of seed treatment. In 1934, the writers (4) published a preliminary note on the subject.

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[2] The writers are indebted to Frances Haglund and R. C. Cassell, graduate students in plant pathology, for their assistance, and to Dr. E. L. LeClerg for help in the statistical work. They wish to express their thanks to Mr. Robert Black, Grain Supervisor, United States Department of Agriculture, Bureau of Agricultural Economics, Minneapolis, Minnesota; Mr. O. A. Zimmerman, Minnesota Board of Grain Appeals, Minneapolis, Minnesota; Dr. LeRoy Powers, Minnesota Agricultural Experiment Station; Dr. L. C. Burnett, Iowa Agricultural Experiment Station; and others for their fine cooperation in supplying lots of barley.

EXPERIMENTAL RESULTS

Microflora of Barley Seed. During 1932–1933 the prevalence of Fusarium and Helminthosporium blight was determined for more than 900 samples of barley obtained from many different localities in the Northwest. In most cases 100 or more seeds were selected at random from each sample, dipped in 70 per cent alcohol, soaked in corrosive sublimate solution for 2 or 3 minutes, and then washed in a solution of 25 to 50 per cent Labarraque sodium hypochloride. The seeds were then placed on acidified potato-dextrose agar in Petri dishes. Many other methods of disinfecting the seeds were tried, but the above method was most satisfactory. Cultures were incubated at room temperature until colonies of fungi developing from the seeds were about 2 cm. or more in diameter. Identifications were then made (Fig. 1).

Several species of bacteria and fungi belonging to more than 25 genera were isolated. Alternaria was the most common fungus, both in 1932–1933 and 1933–1934. Species of this genus, therefore, can be considered the commonest cause of seed blight. Since it was shown, however, by extensive inoculations that *Fusarium* spp. and *Helminthosporium* spp. are the most important of the organisms isolated in causing seedling blight, particular attention was paid to these 2 genera, and the results given in this paper deal mostly with them.

Many different types of Fusarium were isolated, but *Fusarium graminearum* Schwabe was the predominant species. However, in 1933, *Fusarium* spp. belonging to section *sporotrichiella* were rather common. *Helminthosporium sativum* P. K. and B. was by far the most common species of Helminthosporium, although many other species were frequently isolated.

Naturally, there were marked variations in the percentage of seed infected with Fusarium and Helminthosporium. In 1932 the percentage of seeds infected with Fusarium (random selection) varied from 0 to 47 per cent, and Helminthosporium from 0 to 91 per cent. In 1933 the ranges were from 0 to 15 per cent and 0 to 53 per cent, respectively. Seeds with no conspicuous blemish often were infected with these 2 organisms. The average percentage of seeds infected with Fusarium and Helminthosporium in 308 samples in 1932 was 4.1 per cent and 15.1 per cent, respectively (Table 1). In 1933 it was lower, being 3.2 per cent for Fusarium and 13.8 for Helminthosporium, based on 276 samples. The results, given in table 1, also indicate that in 1932 and 1933 *Helminthosporium* spp. were more prevalent than *Fusarium* spp. on blighted seed. In general, seeds infected with Fusarium were infected with Helminthosporium also, the correlation coefficient between relative percentage of Fusarium and Helminthosporium infection for 250 samples in 1932 being 0.989 ± 0.004.

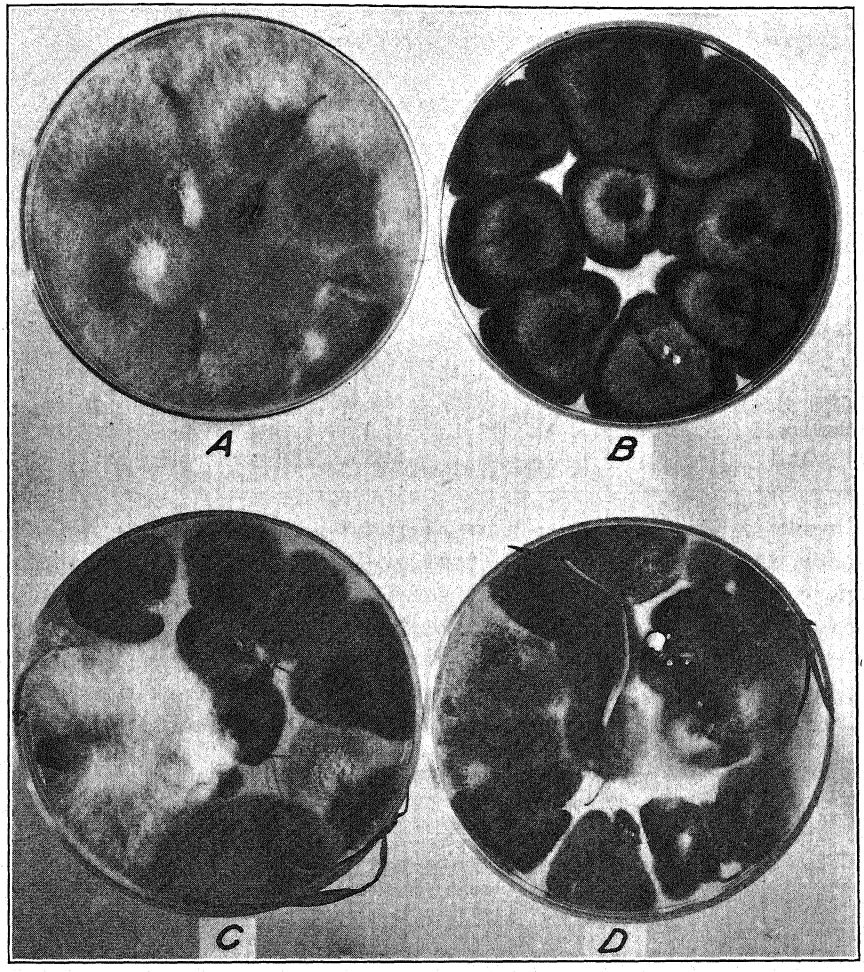


FIG. 1. Colonies of fungi developing from diseased barley seed. A and B. Artificially inoculated with *Fusarium* spp. and *Helminthosporium* spp., respectively. C and D. Naturally infected seed from the Northwest.

There were striking differences in prevalence of these 2 organisms in different regions in the Northwest. In 1932 isolations were made from several varieties, varying from 5 to 9, grown in 15 localities in 6 States. The relative prevalence of Fusarium and Helminthosporium for 10 localities for 3 representative varieties is given in table 2. (See also Tables 5 and 9). It is obvious from table 2 that, even within Minnesota, there were marked differences in percentages of seeds infected with Fusarium and Helminthosporium in different localities. Thus, at Waseca, Glabron had

TABLE 1.—*Prevalence of Fusarium spp. and Helminthosporium spp. on barley seed grown in different localities in the Northwest in 1932–1933*

Year and kind of seed	No. of samples	Percentage of infection			Ratio of Fusarium to Helminthosporium
		Fusarium	Helminthosporium	Total	
1932					
Random	308	4.1	15.1	19.2	1: 3.7
Screenings	79	7.3	21.7	29.0	1: 3.0
Blighted kernels	86	21.1	30.7	51.8	1: 1.5
Blighted kernels	54	24.7	.....	.....	.....
1933					
Random	276	3.2	13.8	17.0	1: 4.3
Blighted	141	7.4	22.3	29.7	1: 3.0

30 per cent, Trebi 28 per cent, and Velvet 9 per cent infection; while at Morris, these same varieties had 5 per cent, 7 per cent, and 6 per cent infection, respectively. The cleanest barley tested in 1932 was obtained from western South Dakota and western North Dakota, while the most severely infected samples were obtained from Iowa and southern Minnesota. Neither Fusarium nor Helminthosporium was isolated from samples obtained from Dickinson, North Dakota, and Highmore, South Dakota, while

TABLE 2.—*The percentage of seeds infected with Fusarium and Helminthosporium in seed lots of 3 varieties of barley grown in 10 localities in 1932*

Place grown	Variety and percentage of infection[a]								
	Glabron			Trebi			Velvet		
	Fus.[b]	Hel.	Total	Fus.	Hel.	Total	Fus.	Hel.	Total
Crookston, Minnesota	3	5	8	1	8	9	0	1	1
Grand Rapids, Minnesota	2	7	9	6	27	33	2	13	15
Morris, Minnesota	2	3	5	2	5	7	0	6	6
Waseca, Minnesota	8	22	30	3	25	28	3	6	9
Fargo, North Dakota	1	2	3	0	4	4	0	5	5
Dickinson, North Dakota	0	0	0	0	0	0	0	0	0
Brookings, South Dakota	4	1	5	0	1	1	0	2	2
Highmore, South Dakota	0	0	0	0	0	0	0	0	0
Kanawha, Iowa	33	36	69	14	54	68	30	28	58
Ames, Iowa	33	25	58	6	91	97	23	25	58
Average	9	10	19	3	22	25	6	9	14

[a] Based on plating out seeds on nutrient agar.

[b] Fus. = *Fusarium* spp.; Hel. = *Helminthosporium* spp.

there was more than 50 per cent infection in those from Iowa (Table 2). Similar results were obtained also from a number of samples from southern Minnesota.

In 1933 the average percentage of barley kernels infected with Fusarium and Helminthosporium was lower than in 1932 (Table 1), although the infection extended over a wider area, and the total amount of seed blight was greater because of the very extensive infection with Alternaria, certain other fungi, and bacteria. Nevertheless, Fusarium and Helminthosporium were not uncommon, the latter being the more prevalent, as was true also in 1932.

Germination and Seedling Injury. In 1932 a study was made to determine the relationship between the percentage of seed infected with Helminthosporium and Fusarium and the following: germination, stand, root rot, seedling blight, stunting, and discoloration of the coleoptile (Table 3).

Germination and root-rot tests were made on moist blotters kept at 25° C. At the end of 7 days the degree of root-rot infection was observed. Seedling injury was studied in the greenhouse. The seed was sown in autoclaved sand in flats, 6 rows of 100 seeds each being sown in each flat. The temperature ranged from 20° C. to 30° C., which is favorable for infection with Fusarium and Helminthosporium. Notes were taken approximately 3 weeks after planting. The results are summarized in tables 3 and 4.

The negative correlation coefficient between percentage of seeds infected and percentage of germination, namely -0.331 ± 0.056, indicates that the higher the percentage of Fusarium and Helminthosporium infection, the poorer the germination. Although there was a significant negative correlation between the degree of infection and the percentage of germination, the difference in germination between clean seed and infected seed often is so slight that this criterion alone is not always a reliable index of the value of the sample for seed. The development of the seedlings also must be taken into consideration, except, of course, in the case of very badly blighted samples.

Special germination tests were made of 118 samples of distinctly blighted seed at 16° C., the temperature of the malting floor. Approximately 69 per cent of the diseased seed germinated, 50 per cent normally, the other 19 per cent weakly or abnormally. The primary rootlet of such seedlings often was stunted, rotted, or aborted; the plumule of some seedlings was contorted, short or even absent, the roots and plumule of others were both injured (Figs. 2 and 3). Ninety-three per cent of the sound seeds selected from these seed lots germinated and nearly all developed strong shoots.

Many of the retarded or weak seedlings that developed on blotters would not have emerged from the soil, while others probably would have been killed a few days after they emerged. This is indicated by the fact that the

TABLE 3.—*Effect of sowing barley seed with different percentages of Helminthosporium and Fusarium infection on germination and seedling injury*[a]

Percentage of infection			Percentage		Number of plants			Degree of root rot
Fusarium	Helmintho-sporium	Total	Germination on blotters	Emergence from sand	With discolored coleoptile	Stunted	Killed	
0	0	0	99	98	11	6	1	10[b]
0	1	1	90	88	3	18	0	30
1	4	6	94	95	14	11	0	10
2	3	4	96	93	3	9	0	10
2	5	7	97	95	24	9	0	10
9	2	11	94	88	16	14	4	30
4	20	24	96	82	37	14	7	40
14	22	36	97	90	24	46	15	50
7	31	38	93	84	38	40	10	40
23	25	48	92	84	28	38	8	50
12	36	48	91	85	18	72	7	80
30	28	58	97	84	50	41	20	30
4	63	67	95	87	11	80	7	60
25	45	70	81	70	59	17	8	30
18	64	82	90	80	37	79	8	90
15	68	83	91	67	29	27	5	70

[a] These are representative samples of approximately 300 barley lots.

[b] Numerals 0–100, in ascending order, indicate relative degree of root-rot infection.

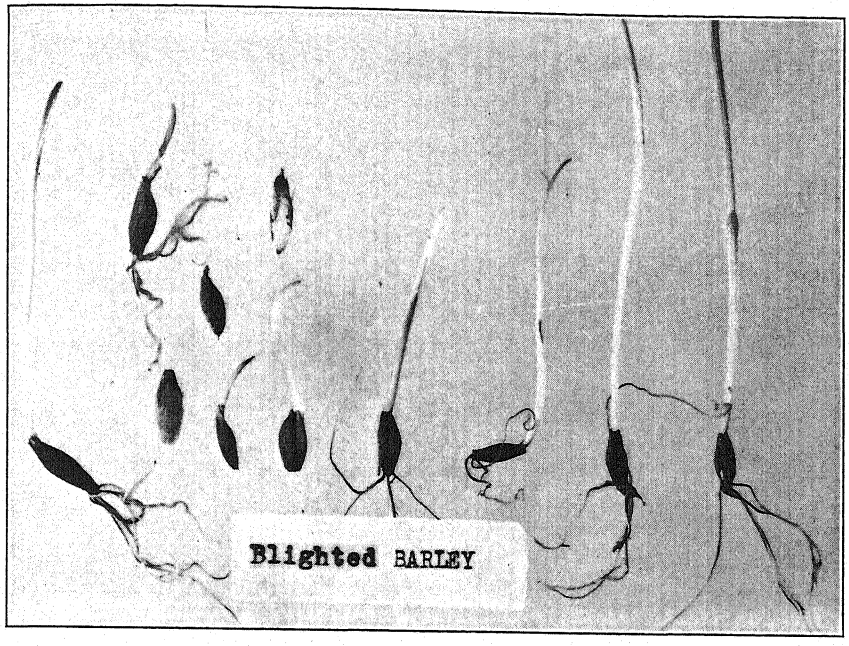


FIG. 2. Germination of blighted seed. Note discoloration of roots and coleoptiles.

average percentage of germination on blotters[3] in 213 samples was 94.5 per cent, but the stand for the same samples sown in sand was only 88 per cent. A correlation coefficient of 0.332 ± 0.068 was obtained between degree of root rot and number of plants stunted. Root infection also is correlated with seed infection ($r = 0.394 \pm 0.065$). This tends to indicate that severity of root infection on blotters is a fairly good index of the development of root rot in soil. Such tests, however, to be reliable, must be made at a relatively high temperature, preferably above 20° C. Many tests were made on blotters at 16°, 20°, and 25° C. Although the seeds were heavily infected with Fusarium and Helminthosporium, practically no root rot developed at 16° C., while at 25° C. the same samples developed severe root rot.

The negative correlation coefficient (-0.507 ± 0.046) between percentage of seeds infected and percentage of plants that emerged proves that Fusarium and Helminthosporium are important in lowering the vitality of barley seed. As one might expect, a high correlation, 0.490 ± 0.048, was obtained between the percentage of germination and percentage of plants that emerged.

[3] The writers are indebted to Mrs. Ruby U. Crouley, Seed Analyst, State Seed Testing Laboratory, University Farm, St. Paul, Minnesota, for making the germination tests reported on blotters, and to Mr. John L. Larson, Assistant Seed Analyst, for his efficient help in the special germination studies.

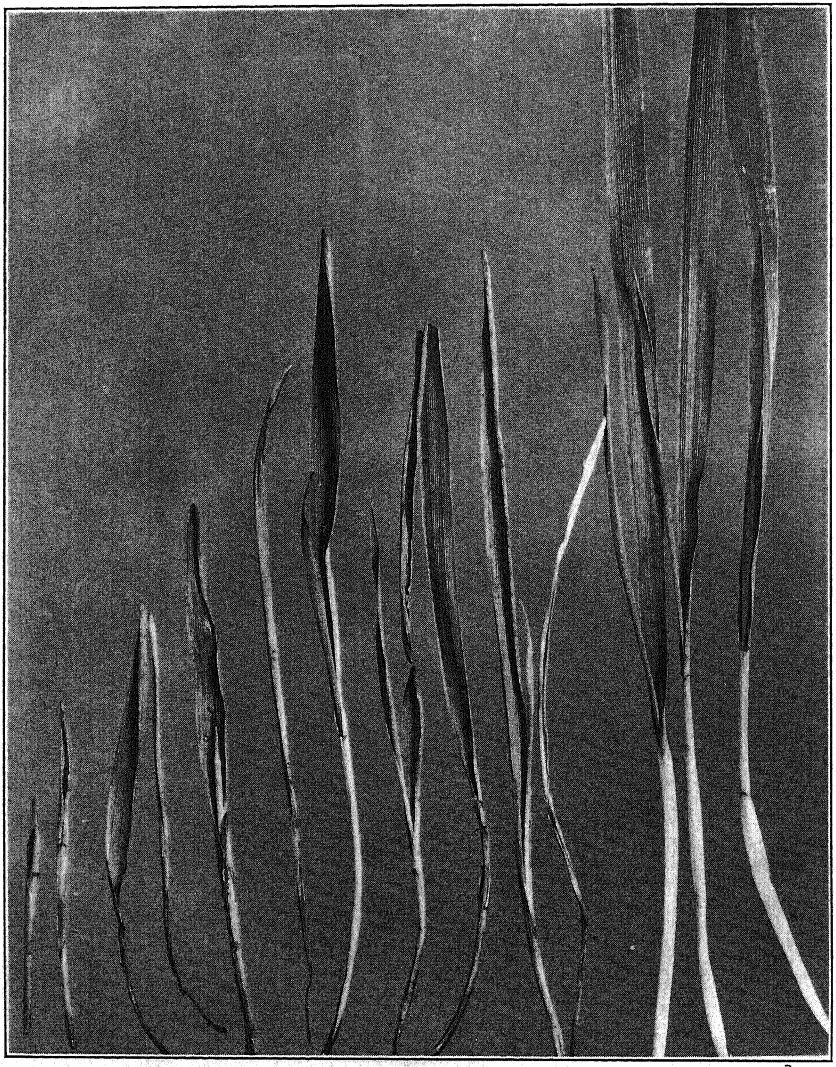


FIG. 3. Diseased seedlings from seed infected with Fusarium and Helminthosporium; three healthy ones on right.

The data given in tables 3 and 4 also indicate that the percentage of seeds infected with Fusarium and Helminthosporium is closely correlated with the following: severity of root-rot infection, (0.394 ± 0.065), discoloration of coleoptile, (0.376 ± 0.057), and number of plants killed, (0.313 ± 0.039). The results indicate that the amount of seedling injury is proportional to the percentage of seeds infected by the 2 pathogens.

When the percentage of seeds infected with Fusarium or with Hel-

TABLE 4.—*Correlation between quality of seed and germination and subsequent development of seedlings*

Number samples compared	Data correlated		Correlation coefficient[a]
250	Percentage seed infected with Fusarium	Percentage infected with Helminthosporium	0.989 ± 0.004
213	Percentage seed infected with Fusarium and Helminthosporium	Percentage seed that germinated	−0.331 ± 0.056
213	do	Percentage plants that emerged	−0.507 ± 0.046
213	Percentage seed that germinated	do	0.490 ± 0.048
116	Percentage seeds infected with Fusarium and Helminthosporium	Degree of root-rot infection (on blotters)	0.394 ± 0.065
234	do	Number of plants with discolored coleoptile	0.376 ± 0.057
234	do	Number of plants stunted or contorted	0.402 ± 0.055
234	do	Number of plants killed	0.313 ± 0.039
234	Percentage seeds infected with Fusarium	Number of plants stunted or contorted	0.288 ± 0.060
234	Percentage seeds infected with Helminthosporium	do	0.382 ± 0.051
168	Degree root-rot infection (on blotters)	do	0.332 ± 0.068

[a] According to Fisher's values for levels of significance of correlation coefficients, all the correlation coefficients surpass the 1 per cent point; therefore, they are highly significant.

minthosporium alone was compared with the number of plants stunted or killed, correlation coefficients of 0.288 ± 0.060 and 0.382 ± 0.051, respectively, were obtained. These figures are lower than that obtained for their combined effect (0.402 ± 0.055). The results indicate that Helminthosporium is more destructive to seedlings than Fusarium, although the correlation coefficient for the latter may have been higher than that obtained had as many seeds been infected with Fusarium as with Helminthosporium. The ratio of the former to the latter in random samples was about 1:4 (Table 1).

Seed treatment. As much of the barley seed produced in Minnesota in many years is likely to be infected with Helminthosporium, Fusarium, and other fungi that cause seedling injury, the effect of seed treatment on the development of seedling blight was tried. Ceresan was selected as the disinfectant because it eliminates barley stripe and might, therefore, be expected to kill other fungi in the glumes and seed coats. The tests were made on 3 varieties of barley from 7 localities. The treated seed was sown

TABLE 5.—*The results of Ceresan treatment of barley seed obtained from seven localities and infected with different amounts of Fusarium and Helminthosporium, when sown in sand in the greenhouse, 1932*

Variety and source of seed	Percentage of seed infected		Percentage emergence		Number of plants							
					Killed		Stunted		Discolored coleoptile		Primary leaf spots	
	Fus.[a]	Hel.	Ck.	Tr.	Ck.	Tr.	Ck.	Tr.	Ck.	Tr.	Ck.	Tr.
Glabron, Minn. No. 445												
Dickinson, North Dakota	0	3	95	94	1	0	5	2	14	0	2	0
Waseca, Minnesota	8	22	82	83	3	0	18	4	45	7	14	0
Winnebago, Minnesota	47	4	77	88	18	3	41	9	82	29	8	1
Ames, Iowa	23	61	74	82	9	2	30	13	77	24	20	7
Minsturdi, Minn. No. 439												
St. Paul, Minnesota	1	3	95	97	1	0	6	3	17	2	2	0
Ames, Iowa	18	64	80	86	13	1	37	6	80	20	21	4
Velvet, Minn. No. 447												
Morris, Minnesota	0	6	88	91	2	1	9	5	15	4	3	0
Waseca, Minnesota	3	6	80	83	13	1	35	8	80	5	14	1
Marshall, Minnesota	32	22	69	84	25	1	55	9	86	21	25	2
Ames, Iowa	23	25	78	81	13	2	41	17	80	4	19	2
Average	16	22	82	87	10	1	28	8	58	12	13	2

[a] Fus. = Fusarium; Hel. = Helminthosporium; Ck. = Check; Tr. = Treated.

in triplicate rows in sterilized sand in flats in the greenhouse. Results are given in table 5.

Ceresan treatment materially increased the stand and decreased number of plants killed, stunted, with discolored coleoptile, and the number with primary lesions. The value of this treatment was associated closely with the degree of seed infection. The higher the percentage of seed infected, the more beneficial the treatment. There was little or no value in treating practically clean seed, *i.e.,* Glabron from Dickinson, North Dakota, or Minsturdi from University Farm, St. Paul. One hundred and eight samples selected at random from the 1933 crop were tested in similar manner except that only a single test was made of each lot. Forty-seven per cent responded to Ceresan treatment and there was no appreciable effect on the remainder.

Most of the seed lots that were not improved by treatment were virtually free from Fusarium and Helminthosporium, although many were heavily infected with other fungi, especially Alternaria. An additional 15 samples of barley free from Fusarium and Helminthosporium and obtained from Washington and Montana also were treated with Ceresan. Not a single sample responded to the treatment. There seems to be little value in treating clean seed if sown in clean soil.

The beneficial effect of treating infected barley seed becomes even more evident when the green weight of seedlings obtained from treated and nontreated seed are compared (Table 6). In this test the barley plants were

TABLE 6.—*Green weight of seedlings grown from treated and nontreated barley seed with different percentages of Fusarium and Helminthosporium infection*

Variety	Per cent seed infected	Mean weight in grams		Per cent increase over check	Differences between means in grams	Differences between means divided by probable error of difference
		Check	Treated[a]			
Glabron	3	11.17 ± 0.32	12.03 ± 0.11	8	0.85 ± 0.34	2.5
do	30	8.60 ± 0.23	12.10 ± 0.48	41	3.50 ± 0.83	4.2
do	50	7.13 ± 0.19	11.97 ± 0.10	68	4.84 ± 0.54	8.9
do	64	6.90 ± 0.03	10.33 ± 0.19	50	3.43 ± 0.19	17.9
Minsturdi	4	15.0 ± 0.52	17.57 ± 0.37	17	2.57 ± 0.64	4.0
do	82	6.17 ± 0.28	13.20 ± 0.56	114	7.03 ± 0.63	11.2
Velvet	6	10.40 ± 0.23	10.87 ± 0.37	5	0.47 ± 0.43	1.1
do	9	6.63 ± 0.25	11.00 ± 0.22	66	4.37 ± 0.32	13.8
do	52	5.63 ± 0.14	10.07 ± 0.20	79	4.44 ± 0.24	18.8
do	48	6.87 ± 0.13	10.63 ± 0.12	55	3.76 ± 0.17	21.7

[a] Treated with Ceresan 3 oz. per bu.

cut off at the ground line when about a month old and weighed immediately. The samples were the same as those listed in table 5. The greatest increases in weight over the checks were obtained from plots sown with the heavily infected seed. For instance, Minsturdi, with 82 per cent of infected seeds, produced 114 per cent more green weight than the control; with 4 per cent infection, the increase was 17 per cent. In both cases the increase was statistically significant. Treating Glabron with 3 per cent and Velvet with 6 per cent infection caused no significant increase in the green weight, but when the percentage of infection was higher there was significant increase.

After obtaining such striking results in the greenhouse, similar tests were made in the field. One hundred and sixteen samples of barley, representing a dozen or more varieties, were selected because of wide variation in degree of infection with Fusarium and Helminthosporium. One portion of the seed was treated with Ceresan, the other was used as control. The treated and nontreated seed was sown in adjoining rows in duplicate series. The test was made on sandy land, previously cropped to rye, near Anoka, Minnesota. Comparative notes on the stand and vigor of growth were taken when the seedlings had developed 2 or 3 leaves and again 3 weeks later. The final percentage figures were based on the average estimates of 3 observers. As each sample had been assigned a code number, all readings were made without knowledge of the kind of seed used. The data thus obtained were correlated with the following: (1) percentage of seeds infected with Fusarium; (2) percentage of seeds infected with Helminthosporium; (3) percentage of seeds infected with both; (4) percentage of plants that emerged in greenhouse; (5) degree of root rotting as determined on moist blotters; (6) number of plants stunted or deformed; and (7) bushel weight of sown grain. The results are summarized in table 7.

All the correlation coefficients listed in table 6 are highly significant except that for bushel weight (0.016 ± 0.093). The results agree in general with those obtained in the greenhouse. They show clearly that poor stand and lack of vigor of seedlings are distinctly associated with increase in percentage of infection in the seed sown. This is particularly noteworthy because the seed was sown in soil cropped the previous year to rye; hence "soil infection" would be expected. But in this experiment seed infection was far more injurious than soil infection. This may be explained, at least partially, on the basis of recent investigations by Henry (5), Sanford and Broadfoot (7), and Bisby, James, and Timonin (1), who have shown that antibiosis of microorganisms of the soil plays a very important rôle in preventing root-rot infection. Preliminary tests at Minnesota indicate that the soil flora has very little effect on preventing root rot and seedling blight resulting from naturally infected seed. It is significant that lightweight, clean seed is better than heavy, infected seed (Tables 6 and 7).

TABLE 7.—*The effect of treating different seed lots of barley with Ceresan, as shown by correlation between the degree of infection in the treated seed and its performance when sown in the greenhouse and field*

Stand and vigor of growth resulting from Ceresan treatment compared with—	Correlation coefficient
Bushel weight	0.016 ± 0.093[a]
Percentage seeds infected with Helminthosporium	0.834 + 0.028
Percentage seeds infected with Fusarium	0.580 ± 0.061
Percentage seeds infected with Fusarium and Helminthosporium	0.826 ± 0.029
Percentage of plants that emerged	− 0.456 ± 0.073
Degree of root infection (on blotters)	0.280 ± 0.084
Number of plants stunted	0.348 ± 0.081

[a] Not significant according to Fisher's values for levels of significance of correlation coefficients, but all the others (correlation coefficients) surpass the 1 per cent point; therefore, they are highly significant.

It seems significant that the correlation between percentage of seeds infected with Helminthosporium alone and the effect of seed treatment was as high (0.834 ± 0.028) as that for combined percentages of Fusarium and Helminthosporium infection and the effects of treatment (0.826 ± 0.029). The correlation between Fusarium infection and the effect of treatment with Ceresan was 0.580 ± 0.061. These high correlations prove that both Fusarium and Helminthosporium are very important in reducing stand and vigor of growth of barley.

Obviously, the kind of seed is important, but only with respect to the degree of infection with fungi (Fig. 4). Table 8 gives the increase in stand and vigor of growth resulting from seed treatment for 7 varieties of barley obtained from 6 localities in Minnesota, 2 in North Dakota, and 2 in Iowa. In Minnesota, the seed lots that responded least to the treatment were from Morris (2.1 per cent infection), while greatest stimulation was in seed from Waseca and Grand Rapids (21 per cent and 19 per cent infection, respectively). The greatest effect of all the treatments was for barley from Ames, Iowa. The 5 varieties of barley from this locality had an average infection of 53 per cent, and the average stand and vigor of growth was increased 54 per cent by the treatment.

The favorable effect of Ceresan on seedlings of barley led to yield trials. Ten different lots of barley with marked variation in percentages of infection with Fusarium and Helminthosporium were treated with Ceresan. The tests were made in 5 sets of duplicate rows, 18 feet long, every other

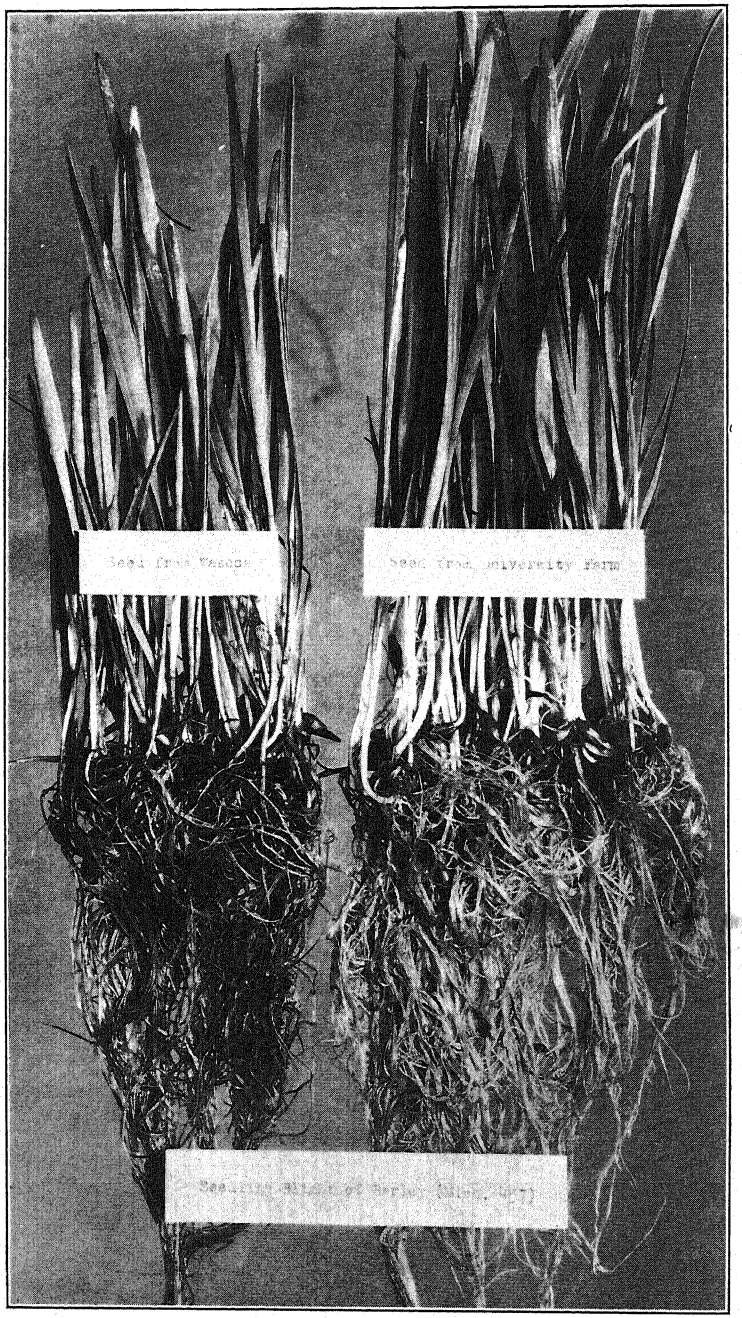


FIG. 4. The relative vigor of seedlings of barley grown from diseased and relatively clean seed. Left, seedlings from seed with 21 per cent infection; right, from seed with 4 per cent infection (Fusarium and Helminthosporium).

TABLE 8.—*The effect of Ceresan on barley seed with different percentages of Fusarium and Helminthosporium infection when sown in the field in 1933*

Variety	Source percentages of infection, vigor, and stand over checks																			
	Waseca, Minn.		Gr. Rapids, Minn.		St. Paul, Minn.		Duluth, Minn.		Crookston, Minn.		Morris, Minn.		Fargo, N. D.		Dickinson, N. D.		Ames, Iowa		Kanawaka, Iowa	
	Inf.[a]	Inc.	Inf.	Inc.	Inf.	Inc.	Inf.	Inc.	Inf.	Inc.	Inf.	Inc.	Inf.	Inc.	Inf.	Inc.	Inf.	Inc.	Inf.	Inc.
Glabron ...	30	0	0	0	7	0	8	0	8	0	0	0	10	0	0	15	84	60	69	40
Manchuria	26	15	18	25	10	55	5	40	4	0	0	0	4	0	0	0	76	95	65	75
Peatland	22	30	10	20	33	0	8	15	5	0	9	0	.....	.....	.....	.....	.....	.....	.....	.....
Svansota	51	35	28	45	7	0	8	0	9	50	4	0	8	0	0	0	.....	.....	.....	.....
Trebi	28	50	33	0	4	10	16	15	9	10	7	0	4	10	0	10	97	105	80	95
Velvet	9	0	15	0	20	20	4	0	1	10	6	15	5	0	0	0	48	50	30	50
Wis. No. 38	26	20	18	15	9	0	11	25	5	10	6	0	6	0	0	0	67[b]	65	20[b]	45
Average	27.4	21.4	18.7	15.0	12.9	12.1	8.6	13.6	5.9	11.4	4.6	2.1	5.3	1.4	0.0	3.6	53.1	53.6	37.7	43.6

[a] Inf. = Percentage of seeds infected with Fusarium and Helminthosporium; Inc. = percentage increase, based on relative stand and vigor of growth in comparison with nontreated seed.

[b] Wis. No. 37 instead of Wis. No. 38.

row being a border. At harvest time 1 foot from each end of the row was discarded. The weight was determined to the nearest gram. The data were analyzed statistically by Student's method. The results are given in table 8. Yields were increased materially by Ceresan treatment, whenever seed lots were heavily infected, except for the variety Glabron. The yield of Velvet from seed with 54 per cent infection was increased 23 per cent, while that of Minn. No. 457 with 21 per cent infection was increased 20 per cent. In general, the percentage of increase resulting from treatment was proportional to the severity of infection. Therefore, the increase probably was due to the effect of Ceresan on the seed-borne pathogens, which were

TABLE 9.—*Yields produced by different seed lots of barley treated with Ceresan and sown in quintuplicate 18 foot rows in the field at University Farm, St. Paul, Minnesota, in 1933*

Kind and source of seed lot	Percentage seeds infected			Increase or decrease in yield per acre[a]		Z[b]	Odds
	Fus.	Hel.	Total	Bu.	Pct.		
Glabron, Minn. No. 445							
St. Paul, Minnesota	2	5	7	+0.8	+ 4.5	.58	5
Waseca, Minnesota	8	22	30	+0.1	+ 0.7	.09	<1
Winnebago, Minnesota	47	4	50[c]	+0.3	+ 1.1	.62	6
Manchuria, Minn. No. 184							
St. Paul, Minnesota	2	8	10	−0.3	− 2.9	.63	6
Waseca, Minnesota	3	24	26[c]	+1.6	+15.1	2.96	500
Minn. No. 457							
St. Paul, Minnesota	1	3	4	+1.3	+ 4.9	1.9	103
Waseca, Minnesota	9	12	21	+5.5	+20.2	12.4	>10,000
Minsturdi, Minn., No. 439							
St. Paul, Minnesota	1	3	4	+0.1	+ 0.4	.09	<1
Velvet, Minn. No. 447							
St. Paul, Minnesota	4	16	20	+3.3	+18.2	2.5	275
Waseca, Minnesota	3	6	9	+3.6	+19.5	6.9	>10,000
Marshall, Minnesota	32	22	52[c]	+4.6	+23.4	9.8	>10,000

[a] As compared with nontreated checks of same seed lot.

[b] Analysis made according to Student's method.

[c] Fusarium and Helminthosporium colonies grew from same seed.

destroyed or at least checked effectively by the fungicide. O'Brien and Dennis (6) obtained similar results with oats infected with *Helminthosporium avenae* in Scotland.

One formaldehyde dust also was tried, but either no increase or an actual decrease in yield resulted. In 1923–1924, a series of tests were made at University Farm on the merit of seed treatment with several fungicides (Ceresan not included) on 4 varieties of barley (3). None increased or decreased the yield. In the light of the results of the present investigations, the negative results may be attributed to the use of comparatively clean seed or ineffective fungicides. It will be noted from table 8 that the yields are rather low. In 1933, it was unusually dry and the apparent differences early in the season between treated and nontreated seed, especially in 2 lots of Glabron, were not substantiated by significant differences in yield. Differences in yield between treated and nontreated seed might be greater in a season of normal rainfall.

The behavior of Glabron toward seed treatment is puzzling. Tisdale, Taylor, and Griffiths (8) concluded that barley varieties are affected differently, with respect to yield, by treating the seed with fungicides. Burnett (2) found that treating seed of Velvet and Glabron with Ceresan did not increase yields, while it increased that of certain other varieties considerably. Under our conditions, however, Velvet responded to seed treatment but Glabron did not.

DISCUSSION

Barley grown in the Northwest is commonly attacked and frequently discolored by a large group of fungi and bacteria. Seeds heavily infected usually are discolored, shrivelled, and of inferior quality. Seedling blight, root rot, basal stem rot, and decrease in yield frequently result when such seed is sown. *Fusarium* spp. and *Helminthosporium* spp. are the most virulent pathogens usually associated with the blighted kernels and with seedling blight.

The results of extensive plating tests show that fungi belonging to more than 25 genera, as well as several kinds of bacteria, cause seed blight in barley. In general, *Helminthosporium* spp. and Alternaria often are more responsible for blighting of the seed than Fusarium. In 1932, for example, Helminthosporium was between 3 and 4 times as prevalent as Fusarium in lots of barley obtained principally from Minnesota and the Dakotas. In 1932 and 1933 Alternaria and bacteria were far more prevalent than either Helminthosporium or Fusarium. It is evident, therefore, that the blighting of barley seed in the spring-wheat region is caused by a group of organisms and that field surveys and plating tests must be resorted to in order to ascertain the relative amount of scab and of blight caused by fungi other than that or those producing the dangerous scab.

Relatively little attention has been paid to the control of the disease complex known as "blight." In fact, relatively little has been known about the suitability of blighted barley for seed.

Laboratory, greenhouse, and field experiments show clearly that there is high correlation between the percentage of seeds infected with *Helminthosporium* spp. and *Fusarium* spp. and the percentage of germination, the stand, the amount of root rot, the number of stunted and deformed plants, and discoloration of the coleoptile. Blighted barley, therefore, is not good seed barley. In some years, however, such as 1933, it would be difficult to obtain good, clean seed barley; hence the question of the effect of seed treatment becomes important.

Treating blighted barley seed may be very beneficial. In the experiments reported in this paper the value of treatment depended on the degree of blighting in the seed, the variety in question, and the fungicide used. As might be expected, the value of treatment was directly proportional to the percentage of infected kernels in the seed lot. For some unknown reason there was no appreciable beneficial result from treating even badly infected seed of Glabron barley. There seem, therefore, to be decided differences in the effect of fungicides on different varieties. Of the fungicides used, Ceresan produced the best results. Formaldehyde dust, on the other hand, not only did not improve stands, but even depressed yield in some cases.

The fact that treating seed with Ceresan usually resulted in better stands and more vigorous plants and that the improvement was proportional to the degree of blighting of the seed indicates that this fungicide, as well as others of the same nature, probably brings about its "stimulatory" effect by killing or inhibiting the deleterious microflora of the seed. It should be emphasized, however, that seed treatment does not always result in increased yields. There are several variables to consider and there may be no beneficial effect from treatment. It is quite clear, however, that yields can often be increased considerably by treating blighted seed or, preferably, by sowing clean seed if it is available.

SUMMARY

During 1932–1934 a study was made of the relative prevalence of Fusarium and Helminthosporium in a large number of samples of Northwestern barley grown in 1932 and 1933 and of the effect on the succeeding crop.

In 1932 Helminthosporium was 3 to 4 times more prevalent than Fusarium. In 1933 the percentage of infected seed was less, but the ratio of Helminthosporium to Fusarium was slightly higher.

In some regions barley was relatively free from "seed blight" in 1932 but was severely blighted in 1933. But most of the discoloration was not primarily due to Helminthosporium and Fusarium but to bacteria and other fungi, especially Alternaria.

Fusarium and Helminthosporium were the common virulent root-rotting organisms associated with diseased barley seed.

There is high correlation between percentage of seed infected with Helminthosporium and Fusarium and the percentage germination of seed, stand, amount of root rot, seedling blight, the number of stunted or deformed plants, and discoloration of coleoptile.

Treating infected seed with Ceresan resulted in significantly better stands and more vigorous growth of seedlings, the improvement being approximately proportional to the degree of infection of the treated seed. Benefits derived from other treatments varied with the severity of seed infection and the fungicide used.

There was no significant correlation between bushel weight of treated seed and the effect of Ceresan treatment.

Seed treatment with Ceresan increased yields, except when the seed was practically clean or when Glabron was used.

There appear to be distinct varietal differences in behavior of barley toward seed treatment, at least as far as yield is concerned. Glabron, although sometimes severely infected, did not respond to seed treatment, while Velvet, Manchuria, and others did.

Infected barley seed treated with Ceresan is not so good as clean, healthy seed.

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A DESCRIPTIVE KEY FOR PLANT VIRUSES

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Research on plant viruses has reached a stage where it is both a challenge and a duty to science to organize such lines of endeavor as can never be strictly a matter of individual concern. The related problems of description, identification, nomenclature, and classification, though they may be particularly difficult subjects upon which to secure general agreement, are, partly for that very reason, in special need of correlated thought and effort. Despite the generally recognized need for an accepted system of description and nomenclature in particular, it appears to be the consensus of opinion that early agreement on such a system is not likely to be secured.

The difficulties in these related problems arise partly from the confusion already existing and partly from the apparently increasing complexity of the subject as further knowledge is gained. The number of distinct plant virus diseases recorded in the literature already exceeds one hundred, and these involve approximately seven hundred plant species. One hundred or more workers are now engaged in the field of plant-virus research in various parts of the world, and there is good reason to believe, therefore, that the number of viruses and hosts reported will continue to increase rapidly for some time. The danger of adding to the present confusion through delay in applying some acceptable system of description is consequently a matter that warrants serious consideration.

Judging from the experience of systematists in other biological fields, the best procedure for a time may be for individuals to contribute such specific suggestions and ideas as they may have on the subject, while at the same time aiming at some form of general agreement through national and international cooperation. It is with this thought and purpose in mind that a tentative scheme for a descriptive key for plant viruses has been drawn up, which is presented and discussed in the following pages. Its greatest value may eventually lie in illustrating the possibilities for the development of a uniform system of description, and in focusing further attention and effort on the problem.

HISTORICAL ASPECTS

The early descriptions of plant virus diseases were naturally chiefly concerned with characteristics that would serve to separate these peculiar maladies from diseases of plants due to other causes. The time had not yet come for attention to distinctions between individual virus diseases. Indeed, the idea that a single type of causal agent might be concerned with

all known forms of plant virus disease prevailed for a considerable time, and on this assumption such distinctions as were noted were accounted for on the basis of the host affected. Even after the description of several different forms of virus disease on a single host (8), true recognition of the existence of a number of specific viruses came only very slowly. This is not surprising in view of the absence of any morphological grounds on which to differentiate the causal agents, and the reliance that was placed on symptomatology for descriptive purposes. The difficulties of differentiation on this basis increased rapidly with the discovery of the influence of host variety and of environmental factors on symptom expression, which, together with complications in terminology and nomenclature, often made identification of a particular disease impossible or uncertain. With the more recent discovery that certain of the better known virus diseases are actually due to the combined action of two or more different viruses, little hope could be maintained for much progress in separating the increasing number of diseases recognized on the criterion of symptoms alone.

Consequently, although the differentiation of virus diseases such as those of the potato on the basis of symptom expression served its purpose very well in the earlier periods of study, it now appears that this method is of only limited application to plant virus diseases as a group. Aside from the specific criticisms of inadequacy and unreliability, which may be offered against this system, the principle itself is faulty from the point of view of modern pathology. It is to be regretted, therefore, that much of the more recent confusion in literature on virus diseases is attributable partly to their continued description on the basis of symptomatology where such appears to be no longer necessary nor desirable.

Description and identification of the virus itself, whenever possible, on the basis of its behavior and reactions (4, 5, 6, 9) offers far greater opportunities than is generally suspected, and is furthermore likely to remain the only satisfactory means of differentiation. This principle has now been given sufficient trial to warrant general assurance of its reliability, and to a considerable extent has been found adequate. Adoption of the principle of describing the virus rather than the disease does not eliminate the usefulness of symptom descriptions as a valuable adjunct, but merely reduces the emphasis on this feature in cases where it is neither reliable nor necessary. The plan presented in this paper for the description of plant viruses proposes, in fact, to use for differential purposes any or all characteristics of the virus or of the resultant disease according as need arises.

THE BASES FOR DESCRIPTION OF A PLANT VIRUS

From the point of view of identification, the simplest and most concise description that will suffice to distinguish one virus from any other will

prove the most satisfactory. Those who are acquainted, through repeated observation or research, with a particular virus disease may regard even a very elementary description of the virus itself as superfluous, since the disease symptoms may be regarded as entirely sufficient for recognition. This criticism, however, may have application to only relatively few viruses, and, even in such cases, a fundamental description may often serve to place specialists in a particular virus on guard for new forms. Certainly, a long acquaintance with ordinary tobacco mosaic would not qualify anyone to make a reliable determination by inspection only.

On the other hand, the introduction of minute details of behavior or properties of a virus, which are unnecessary or determinable only with difficulty, should not be included in a description merely because these points are known. Nevertheless, such features may eventually become significant for descriptive purposes. For the present, however, it has not been found necessary or advisable to utilize such characteristics as filterability, tolerance to dilution, resistance to chemicals, serological reactions, adsorption relations, influence of environment on symptoms, distribution of virus in host, or many other details of changes of a macroscopic or microscopic character.

In the light of present knowledge, the features of paramount value for the description and identification of plant viruses are considered to be: (1) Modes of transmission, including the identity of any known insect vector; (2) natural and differential hosts; (3) longevity *in vitro*; (4) thermal death point; (5) certain distinctive or specific symptoms.

The various modes of transmission of a plant virus, in addition to forming distinctions of major importance, may also serve for more specific purposes. This is particularly true of transmission by sucking insects, for not only does the type of insect, *i.e.*, aphid, leaf hopper, thrips, white fly, form an important distinction, but the specific vector concerned is in many instances the main criterion for identification. Transmission by plant extract, tissue insertion, or grafting, or through vegetative parts, seed, or soil complete the possible means by which a virus may be spread. The use of natural or differential hosts as a means of determination constitutes a useful but often less reliable criterion than the modes of transmission, unless considered in conjunction with insect relations, virus properties, or specific symptoms.

DEVELOPMENT OF THE KEY

When the various characters discussed above are established, within the limits of possibility, for individual viruses, it becomes possible by different methods to separate the viruses into several fairly well-defined groups. As these are studied in more detail, it becomes apparent that some degree of

natural relationship exists within certain groups. Hence, in the further development of such groupings into a descriptive key for plant viruses, the question of possible natural relationships must influence the choice of the system to be followed. Furthermore, in the creation of divisions and subdivisions of the different virus groups in a dichotomous system to form such a key, great importance must be attached to the criteria or bases on which the first distinctions are made. The relationship of sucking insects to plant viruses constitutes perhaps the most natural and fundamental line of separation (1, 6, 9). Thus the viruses may be separated first into those that are, and those that are not, transmissible by some type of sucking insect. The latter group, depending as it does on negative results of experimental tests, may, to be sure, eventually prove to contain many viruses that must be transferred to the former group, and it may finally develop, as is now suspected by some, that all viruses are transmissible by some sucking insect. In such event, this first criterion for separation will naturally be eliminated and the subsequent criteria, especially the identity of the vector involved, will increase in relative significance.

Everything considered, it has seemed most satisfactory to base the second criterion of distinction on mechanical transmissibility of the virus by plant extract. It might be maintained that second rank should be allotted to the type of insect vector concerned. Nevertheless, it is considered preferable to sacrifice this choice for the desired sequence in subsequent subdivisions, intended to be based largely upon certain properties of the viruses *in vitro*. Accordingly, the identity of the insect vector has been used as a subsequent criterion of differentiation, and it attains special significance in those subgroups where mechanical transmission by plant extract fails. For the convenience of the reader, the chief subdivisions used in the key are shown in schematic form in table 1.

In mechanical transmission by plant extract, two criteria are involved, namely, that plant extract must yield infection and that infection must be accomplished by simple or ordinary methods of inoculation, such as needle punctures, rubbing, or injection. Mechanical insertion of pieces of tissue, with or without union of cells, and infection from plant juice through the medium of insects have been excluded from this category. For the present, also, it has seemed advisable to exclude all rare, doubtful, or very difficult cases of transmission by means of plant extract. Separate groups for viruses transmitted by more unusual methods, such as the insertion of pieces of tissue (as distinguished from true grafting), have not been included, because these modes of transmission are not regarded as fundamental in importance, nor are they always easily accomplished with certainty.

The striking range in longevity *in vitro* and in thermal death point of viruses that are transmissible mechanically by plant extract has suggested

TABLE 1.—*The chief groups of plant viruses*

The Plant Viruses	Transmissible by sucking insects (A_1)	Not transmissible mechanically by plant extract (B_1)	Not transmissible by aphids (C_1)	Transmissible by leaf hoppers (D_1)
				Not transmissible by leaf hoppers (D_2)
			Transmissible by aphids (C_2)	Rosaceae susceptible (D_1)
				Rosaceae not susceptible (D_2)
		Transmissible mechanically by plant extract (B_2)	Longevity *in vitro* less than 7 days at 22° C. (C_1)	Thermal death point below 60° C., 10 min. (D_1)
				Thermal death point 60° C., or above, 10 min. (D_2)
			Longevity *in vitro* 7 days or more at 22° C. (C_2)	Thermal death point below 80° C., 10 min. (D_1)
				Thermal death point 80° C., or above, 10 min. (D_2)
	Not transmissible by sucking insects (A_2)	Transmissible mechanically by plant extract (B_1)	Longevity *in vitro* 7 days or more at 22° C. (C_1)	Thermal death point 60° C., or above, 10 min. (D_1)
				Thermal death point less than 60° C., 10 min. (D_2)
			Longevity *in vitro* less than 7 days at 22° C. (C_2)	Thermal death point 60° C., or above, 10 min. (D_1)
				Thermal death point less than 60° C., 10 min. (D_2)
		Not transmissible mechanically by plant extract (B_2)	Transmissible by grafting (C_1)	Potato susceptible (D_1)
				Potato not susceptible (D_2)
			Not transmissible by grafting so far as known (C_2)	Transmissible by other vegetative means (D_1)
				Not transmissible by other vegetative means (D_2)

the employment of these features in the differentiation of certain groups. Some degree of natural relationship appears to be reflected in the behavior of certain viruses toward these tests; though the points of separation selected in the present scheme are, of course, purely arbitrary.

Separation on the basis of longevity of extracts *in vitro* offers many possibilities. For the sake of simplicity and convenience, however, only two subdivisions have been made, namely, to include: (1) viruses whose longevity *in vitro* is less than 7 days and (2) those that can withstand aging for 7 days or more. Since the temperature at which the extracts are stored greatly influences the longevity of a virus, a standard temperature should be selected for this test. On the whole, room temperature (about 22° C.) may prove most suitable and convenient. While this temperature may be more difficult to maintain at a constant level in some laboratories than higher or lower temperatures, the majority of workers should find it the easiest to approximate without special equipment. The 7-day period for aging is suggested partly because it is a convenient length of time, and partly because it serves to separate, among others, viruses of the cucumber mosaic type from certain forms of greater longevity.

The critical thermal-death-point temperatures selected are those of 60° and 80° C. In the present key, a mechanically-transmitted virus will fall into one of several categories, according to its grouping in the preceding classes and whether or not its thermal death point is above or below either 60° or 80° C. (Table 1). Thus, unless the thermal death point of a virus lies close to the critical temperature, it may not be necessary to make a very accurate determination for purposes of identification. A reasonable degree of standardization of methods is essential in such tests of properties. Everything considered, it seems simplest in the longevity and thermal-death-point determinations to use undiluted extract or extract diluted only 1 to 1. Most of the thermal death points accepted for the present key are based upon 10-minute exposures of 1 or 2 cc. of plant extract in thin-walled test tubes. The host from which the virus is extracted does not appear markedly to influence the results secured, and, while in most cases it may be best to use the natural or most common host, in other cases the determination may be of greater value if made upon certain susceptible hosts that are known to be affected by other related viruses.

The criterion of transmission by grafting, used in one section of the key, is not always a simple one in practice, especially in the case of monocotyledons or herbaceous dicotyledons. For the present, however, it must remain an important diagnostic feature for certain viruses, even though negative results may be inconclusive, for the reason that transmission in this manner may constitute the only proof yet available of the virus nature of particular diseases to be included in this classification.

The diagnostic value of natural and differential hosts is obviously great in many instances. Nevertheless, it is clear from the narrow host ranges of some viruses and the wide and overlapping host ranges of others that, in general, only a limited relationship may be concluded to exist between plant viruses and the different plant families. Description of a virus on a single host only, or on a limited group of hosts, without regard to the value of these for differential purposes, has often led to synonomy or to confusion in nomenclature.

Although, as has been pointed out, symptom expression may be unreliable or of limited value for diagnostic purposes when the specific virus concerned is to be determined, it nevertheless seems desirable to include some reference to symptoms in the descriptions of all viruses. In the case of those transmitted only with difficulty or not at all by plant extract, symptom expression sometimes constitutes one of the chief diagnostic characters. The undesirable features of symptomatology for descriptive purposes relate largely to the use of and reliance upon minute details not in themselves sufficiently characteristic or distinctive to be of differential value. For present purposes, description of symptoms may be confined chiefly to such terms as mottling, chlorosis, malformation and necrosis, when other more fundamental characters of the virus also are given.

Other criteria may be useful on rare occasions for distinguishing particular forms, as, for instance, transmissibility through seed or through soil, and the presence of intracellular inclusions, or x-bodies. With the discovery of an increasing number of "strains" of certain viruses, still other diagnostic characters will no doubt need to be introduced, or present ones expanded, in order to meet future requirements.

The descriptive key here presented has been developed according to these considerations (Table 3). Actually, it has been possible to place about 50 forms in their respective positions in the system. This number, representing about one-third to one-half of the reported viruses, is believed to be sufficiently inclusive to illustrate most of the virus types or "type-forms." The omission of certain apparently well-described viruses is in many cases due to the lack of reliable information on one of the specific diagnostic features, as, for instance, the insect relations. Sufficient information is not yet available on a large percentage of plant viruses to allow them to be definitely placed in the key, and a considerable number must consequently remain in the undetermined group until the necessary information has been secured. While this group need not concern us greatly in the present connection, it has seemed worth while to list most of these viruses in such a way as to indicate some of the chief diagnostic features that are lacking (Table 2).

As an example of incomplete description may be cited the senior writer's

TABLE 2.—*A list of some incompletely described viruses, showing the chief undetermined or doubtful diagnostic features*

+ determined; ? undetermined or doubtful

Transmission by			Properties	Virus of
Sucking insects	Plant extract	Grafting		
+	+		?	clover mosaic crucifer mosaic Easter-lily mosaic iris mosaic lettuce mosaic potato internal-parenchyma necrosis potato spindle tuber potato unmottled curly dwarf sugar-cane mosaic tulip breaking
?	+		+	dock mosaic tobacco bleaching mosaic tobacco mild mosaic tobacco speckled mosaic tomato stem necrosis
?	+		?	anthurium mosaic cowpea mosaic delphinium mosaic grape mosaic hop chlorosis lily-of-the-valley mosaic narcissus gray disease petunia mosaic potato aucuba mosaic potato calico potato paracrinkle rose wilt soy-bean mosaic sweet-potato mosaic tobacco etch tobacco Rotterdam-B disease tomato bunchy top vegetable-marrow mosaic wheat rosette white-clover mosaic
?	?	+		apple mosaic black-currant reversion black-locust brooming disease cherry buckskin citrus chlorosis dahlia ring spot fig mosaic hop mosaic hop nettlehead peach little peach peach mosaic peach phony disease peach red suture pelargonium mosaic

TABLE 2.—(*Continued*)

Transmission by			Properties	Virus of
Sucking insects	Plant extract	Grafting		
?	?	+		plum pox potato apical leaf roll potato interveinal mosaic potato supermild mosaic potato yellow top rose chlorosis spindle-tree chlorosis tomato big bud tomato witches' broom vine court-noué
?	?	?		anemone alloiophylly bean rugose mosaic dahlia oakleaf gladiolus mosaic hippeastrum mosaic potato giant hill raspberry streak rubber brown bast sweet-potato mosaic violet mosaic

account of several viruses on tobacco (4), which disregards possible insect relations. Similarly, E. M. Johnson's description of virus diseases of tobacco in Kentucky (3) omits certain essentials for purposes of adequate description and identification. It is in such instances, where transmissibility by plant extract is known but property determinations are lacking, or where transmissibility by grafting is determined but insect relations uncertain, that the largest groups of imperfectly described viruses fall. It is hoped that, as virologists find opportunity to work with viruses whose descriptions are yet incomplete, the necessary effort to obtain the desired information will be made.

As an illustration of what may be considered a sufficiently complete description of a virus transmissible mechanically by plant extract, the following example may be given:

Ordinary tobacco mosaic virus (*tobacco virus* 1). Type: Allard, H. A., Jour. Agr. Research **6**: 649–674. 1916. Transmissible with difficulty by aphids (*Myzus pseudosolani* Theob. and others) from tomato (*Lycopersicon esculentum* Mill.), but not from tobacco. Readily transmitted mechanically by plant extract. Longevity *in vitro*, 3 months or more. Thermal death point 92° C., 10 minutes. Tobacco (*Nicotiana tabacum* L.) and many other solanaceous and non-solanaceous plants susceptible. Necrotic local lesions on *Nicotiana glutinosa* L. Inclusion bodies (x-bodies and striated crystals) present in tobacco and many other hosts. Varying shades

of green coarse mottling on tobacco. Attenuated strains and other strains, separable chiefly on basis of symptoms, occur in nature. Distribution coexistent with tobacco culture.

DISCUSSION

The placing of the viruses in the key has been influenced to some extent by individual judgment as to what may or may not be accepted as adequate determination of any particular character. The element of error in the position assigned to some forms may, therefore, be considerable, and we may reasonably expect to find certain viruses needing to be shifted from time to time, following further investigations.

As far as is known, no previous serious attempt has been made to bring all known forms of plant virus diseases together in related groups, although several suggested classifications have been made for such groups of virus diseases as those of the potato (2, 7), and of tobacco (3). The greatest weakness of these systems has been the lack of applicability of the principles involved to larger groups of known virus diseases. Such broader groupings, based upon symptom expression, as "yellows" and "mosaic," are also no longer satisfactory as a starting point for differentiation.

It may be argued that in the absence of any morphological grounds differentiation and description of the virus itself is impractical. Considered from a broad point of view, the progress made in the recognition of such invisible entities as atoms, vitamins, and genes should be encouraging. More specifically, however, in the case of the viruses that are readily transmitted by plant extract, we have means of description that are quite as reliable and satisfactory as those available in the case of many morphologically related forms of bacteria and fungi, where behavior and reaction to specific conditions are basic to differentiation. In the case of viruses not transmissible by plant extract, this characteristic itself serves in part as an advantage in that it excludes a large group which might otherwise need to be separated by more artificial and complicated methods. Distinctions in this latter group, fortunately, may usually be accomplished by means of insect vectors.

One of the most promising fields for research on plant viruses relates to the study of the similarities and relationships between various viruses and groups of viruses. In the earlier period of research, the similarity of different forms was stressed; more recently their dissimilarity has been emphasized. The present period of investigation is already tending strongly toward the recognition of different "strains" or forms of one virus, closely related in their more fundamental characters, but differing in minor features or in symptom expression. Hence, virus research has already reached the stage where studies on a particular virus that disregard

TABLE 3.—*A descriptive key for plant viruses*

A_1. Transmissible by sucking insects.
B_1. NOT TRANSMISSIBLE MECHANICALLY BY PLANT EXTRACT.
C_1. Not transmissible by aphids.
D_1. Transmissible by leaf hoppers.
E_1. China aster (*Callistephus chinensis* Nees) susceptible.
Vein clearing; chlorosis of leaves and flowers; proliferation.
Transmitted by *Cicadula sexnotata* Fall.
ASTER YELLOWS VIRUS

E_2. Cranberry (*Vaccinium macrocarpon* Ait.) susceptible.
Malformation of flowers; proliferation of lateral buds.
Transmitted by *Euscelis striatulus* Fall.
CRANBERRY FALSE-BLOSSOM VIRUS

E_3. Peach (*Prunus persica* Sieb.) susceptible.
Chlorosis and necrosis of foliage; proliferation of lateral buds.
Transmitted by *Macropsis trimaculata* Fitch.
PEACH YELLOWS VIRUS

E_4. Sandal (*Santalum album* L.) susceptible.
Chlorosis, proliferation and necrosis; malformation of floral parts.
Transmitted by *Moonia albimaculata* Distant.
SANDAL SPIKE VIRUS

E_5. Sugar beet (*Beta vulgaris* L.) susceptible.
Vein clearing, leaf rolling, vein enations, phloem necrosis.
Transmitted by *Euttettix tenella* Baker.
SUGAR BEET CURLY TOP VIRUS

E_6. Corn (*Zea mays* L.) susceptible.
F_1. Inclusion bodies present.
Mottling along veins.
Transmitted by *Peregrinus maidis* Ashm.
CORN MOSAIC VIRUS

F_2. No inclusion bodies present.
Mottling along veins.
Transmitted by *Cicadulina mbila* Naude.
CORN STREAK VIRUS

E_7. Sugar cane (*Saccharum officinarum* L.) susceptible.
Galls on leaves and stalks.
Inclusion bodies present.
Transmitted by *Perkinsiella vastatrix* Breddin and *P. saccharicida* Kirk.
SUGAR-CANE FIJI-DISEASE VIRUS

E_8. Rice (*Oryza sativa* L.) susceptible.
Mottling and stunting.
Inclusion bodies present.
Transmitted by *Nephotettix apicalis* Motsch.
RICE STUNT-DISEASE VIRUS

D_2. Not transmissible by leaf hoppers.
E_1. Transmissible by thrips.
Pineapple (*Ananas sativus* L.) susceptible.
Chlorotic spots and concentric rings; necrosis.
Transmitted by *Thrips tabaci* Lind.
PINEAPPLE YELLOW-SPOT VIRUS

E_2. Not transmissible by thrips.
F_1. Transmissible by white fly.
G_1. Tobacco (*Nicotiana tabacum* L.) susceptible.
Vein thickening and enations; leaf curling.
Transmitted by *Bemisia* sp.
TOBACCO LEAF-CURL VIRUS

G_2. Cotton (*Gossypium herbaceum* L.) susceptible.
Vein thickening and enations; leaf curling.
Transmitted by *Bemisia gossypiperda* Misra & Lamba.
COTTON LEAF-CURL VIRUS

F_2. Not transmissible by white fly.

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TABLE 3.—(*Continued*)

C_2. Transmissible by aphids.
  D_1. Rosaceae susceptible.
    E_1. Raspberry (*Rubus strigosus* Michx.) susceptible.
      F_1. Transmitted by *Amphorophora rubi* Kalt.
        G_1. Mottling, necrosis, and malformation.
          RED RASPBERRY MOSAIC VIRUS
        G_2. Yellow mottling, chlorosis, necrosis, and malformation.
          RASPBERRY YELLOW MOSAIC VIRUS
      F_2. Transmitted by *Aphis rubiphila* Patch.
        Leaves dark green, sometimes bronzed, curled.
        G_1. Black raspberry not susceptible.
          RASPBERRY LEAF-CURL VIRUS (ALPHA TYPE)
        G_2. Black raspberry susceptible.
          RASPBERRY LEAF-CURL VIRUS (BETA TYPE)
    E_2. Blackberry (*Rubus* spp.) susceptible.
      Mild mottling and dwarfing.
      Transmitted by *Capitophorus tetrarhodus* Walker.
        BLACKBERRY DWARF VIRUS
    E_3. Strawberry (*Fragaria* spp.) susceptible.
      Transmitted by *Myzus fragaefolii* Ckll.
      F_1. Chlorosis and malformation; no proliferation.
        STRAWBERRY XANTHOSIS VIRUS
      F_2. Numerous spindling petioles.
        STRAWBERRY WITCHES'-BROOM VIRUS
    E_4. Ocean spray (*Holodiscus discolor* Max.) susceptible.
      Brooming and dwarfing.
      Transmitted by *Aphis spiraeae* Schout.
        OCEAN-SPRAY WITCHES'-BROOM VIRUS
  D_2. Rosaceae not susceptible so far as known.
    E_1. Potato (*Solanum tuberosum* L.) susceptible.
      Transmitted by *Myzus persicae* Sulz.
      F_1. Leaf rolling, chlorosis, brittleness, phloem necrosis.
        POTATO LEAF-ROLL VIRUS
      F_2. Chlorosis; necrosis of pith in stem and tuber.
        POTATO YELLOW-DWARF VIRUS[a]
    E_2. Dahlia (*Dahlia variabilis* Desf.) susceptible.
      Mottling, malformation, often vein necrosis.
      Inclusion bodies present.
      Transmitted by *Myzus persicae* Sulz.
        DAHLIA MOSAIC VIRUS
    E_3. Banana (*Musa cavendishii* Lamb.) susceptible.
      Mottling, malformation, and necrosis.
      Transmitted by *Pentalonia nigronervosa* Coq.
        BANANA BUNCHY-TOP VIRUS
    E_4. Peanut (*Arachis hypogaea* L.) susceptible.
      Chlorosis, stunting, and mottling.
      Transmitted by *Aphis leguminosae* Theob.
        PEANUT ROSETTE VIRUS
    E_5. Alfalfa (*Medicago sativa* L.) susceptible.
      Mottling, sometimes ring-like.
      Transmitted by *Illinoia* (*Macrosiphum*) *pisi* (Kalt.) Baker.
        ALFALFA MOSAIC VIRUS
    E_6. Garden pea (*Pisum sativum* L.) susceptible.
      Mottling and malformation.
      Transmitted by *Illinoia* (*Macrosiphum*) *pisi* (Kalt.) Baker.
        PEA MOSAIC VIRUS
    E_7. Easter lily (*Lilium longiflorum* Baker) susceptible.
      Chlorosis and downward curling of leaves.
      Transmitted by *Aphis gossypii* Glov.
        EASTER-LILY YELLOW FLAT VIRUS

[a] Also reported as transmitted by a leaf hopper (*Agallia sanguinolenta* Prov.).

TABLE 3.—(*Continued*)

B_2. TRANSMISSIBLE MECHANICALLY BY PLANT EXTRACT.
C_1. Longevity *in vitro* less than 7 days at 22° C.
D_1. Thermal death point below 60° C. (10 minutes).
E_1. Not transmissible by aphids.
F_1. Transmissible by thrips.
Transmitted by *Frankliniella insularis* Franklin and *Thrips tabaci* Lind.
Tomato (*Lycopersicon esculentum* Mill.) susceptible.
Bronzing, ring chlorosis, and necrosis.
TOMATO SPOTTED WILT VIRUS

E_2. Transmissible by aphids.
F_1. Tobacco (*Nicotiana tabacum* L.) not susceptible.
G_1. Potato (*Solanum tuberosum* L.) susceptible.
H_1. Mild mottling, slight wrinkling.
POTATO MILD MOSAIC VIRUS
H_2. Pronounced mottling and wrinkling.
POTATO CRINKLE MOSAIC VIRUS
G_2. Potato (*S. tuberosum* L.) not susceptible.
Bean (*Phaseolus vulgaris* L.) susceptible.
H_1. Transmissible through seed.
Mottling and malformation.
BEAN MOSAIC VIRUS
H_2. Not transmissible through seed.
Intense yellow mottling and malformation.
BEAN YELLOW MOSAIC VIRUS
F_2. Tobacco (*N. tabacum* L.) susceptible.
Sugar beet (*Beta vulgaris* L.) susceptible.
Mottling and malformation.
SUGAR-BEET MOSAIC VIRUS
D_2. Thermal death point 60° C. or above (10 minutes).
E_1. Transmissible by aphids.
F_1. Celery (*Apium graveolens* L.) susceptible.
G_1. Watermelon (*Citrullus vulgaris* Schrad.) susceptible.
Mottling and necrosis on celery.
CELERY MOSAIC VIRUS
G_2. Watermelon not susceptible.
Mottling, necrosis, and malformation on cucumber (*Cucumis sativus* L.)
CUCUMBER MOSAIC VIRUS
F_2. Potato (*Solanum tuberosum* L.) susceptible.
G_1. Tobacco (*Nicotiana tabacum* L.) susceptible.
Mild veinbanding symptoms on tobacco.
POTATO VEINBANDING VIRUS
G_2. Tobacco (*N. tabacum* L.) not susceptible.
Mottling; rolling of upper leaves on potato.
POTATO LEAF ROLLING MOSAIC VIRUS
F_3. Onion (*Allium cepa* L.) susceptible.
Chlorosis and malformation.
ONION YELLOW DWARF VIRUS
E_2. Not transmissible by aphids.

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C_2. Longevity *in vitro* 7 days or more at 22° C.
D_1. Thermal death point below 80° C. (10 minutes).
E_1. Transmissible by aphids.
Alfalfa (*Medicago sativa* L.) susceptible.
Mottling on alfalfa; local necrosis on bean (*Phaseolus vulgaris* L.)
ALFALFA VIRUS 2
E_2. Not transmissible by aphids.

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D_2. Thermal death point 80° C. or above.

TABLE 3.—(*Continued*)

E_1. Transmissible by aphids from tomato (*Lycopersicon esculentum* Mill.).
Tobacco (*N. tabacum* L.) susceptible.
Local necrosis on *Nicotiana glutinosa* L.
Inclusion bodies present.
F_1. Green coarse mottling pronounced on tobacco.
ORDINARY TOBACCO MOSAIC VIRUS
F_2. Mild or no mottling on tobacco.
ATTENUATED TOBACCO MOSAIC VIRUS
F_3. Yellow mottling on tobacco.
YELLOW TOBACCO MOSAIC VIRUS
E_2. Not transmissible by aphids from tomato.
* * * * * *

A_2. Not transmissible by sucking insects.
B_1. TRANSMISSIBLE MECHANICALLY BY PLANT EXTRACT.
C_1. Longevity *in vitro* 7 days or more at 22° C.
D_1. Thermal death point 60° C. or above (10 minutes).
Tobacco (*N. tabacum* L.) susceptible.
E_1. Tomato (*Lycopersicon esculentum* Mill.) susceptible.
F_1. Faint or mild mottling on tobacco.
POTATO MOTTLE VIRUS
F_2. Small necrotic rings on tobacco.
POTATO RING SPOT VIRUS
E_2. Tomato (*L. esculentum* Mill.) not susceptible (*i.e.*, to juice inoculation).
Large necrotic rings on tobacco (*N. tabacum* L.).
TOBACCO RING SPOT VIRUS
D_2. Thermal death point less than 60° C. (10 minutes).
* * * * * *
C_2. Longevity *in vitro* less than 7 days at 22° C.
* * * * * *
B_2. NOT TRANSMISSIBLE MECHANICALLY BY PLANT EXTRACT.
C_1. Transmissible by grafting.
D_1. Potato (*Solanum tuberosum* L.) susceptible.
E_1. Yellow blotches on leaves.
POTATO AUCUBA MOSAIC VIRUS
E_2. Brooming; dwarfing of leaves and tubers.
POTATO WITCHES'-BROOM VIRUS
D_2. Potato (*S. tuberosum* L.) not susceptible.
E_1. Abutilon (*Abutilon theophrasti* Medic.) susceptible.
Mottling or variegation of leaves.
ABUTILON MOSAIC VIRUS
E_2. Peach (*Prunus persica* Sieb.) susceptible
Chlorosis and rosetting of foliage.
PEACH ROSETTE VIRUS
C_2. Not transmissible by grafting so far as known.
D_1. Transmissible by other vegetative means.
E_1. Potato (*Solanum tuberosum* L.) susceptible.
Gigantism of vines and tubers, no mottling.
POTATO GIANT HILL VIRUS
E_2. Raspberry (*Rubus strigosus* Michx.) susceptible.
Leaf curling and blue stripes on canes.
RASPBERRY STREAK VIRUS
D_2. Not transmissible by other vegetative means.
Not transmissible by seed.
Not transmissible through soil.
Cotton (*Gossypium herbaceum* L.) susceptible.
Malformation, sterility, no mottling.
COTTON CRAZY TOP VIRUS

the probable relationships of other strains or forms are handicapped at the outset. Although a fairly clear conception of many groups of viruses has now come to be formed, the relation of these groups to one another has not yet been given much consideration. With the wealth of material available, investigations aimed primarily at studying such relationships are likely to yield some most interesting results of taxonomic value. While it may yet be too early to attempt a sound classification scheme for plant viruses based upon "natural" relationships, the possibilities in this direction are perhaps greater than is generally supposed. Viruses already recognized perhaps represent the extremes of a variety of types between which may be found a remarkable gradation of forms. The ordinary tobacco-mosaic virus, for instance, may be taken as a "mutating" form. With respect to symptom expression, it is known to "mutate" in nature and it may be altered in certain respects by artificial means. It is, or it is not, transmitted by aphids according to the host used as a source of the virus. Yet, as far as its properties are concerned, the ordinary tobacco-mosaic virus is remarkably stable, and the stability of the various "strains" of this virus also is well established. Compared to other known viruses, these and other characteristics suggest that the ordinary tobacco-mosaic virus is probably an intermediate form, and does not represent a natural extreme. It may be futile to speculate as to which forms represent the extremes of a theoretical evolutionary scale, but without attempting to indicate which might be regarded as the highest and most specialized type and which the lowest or most primitive, it is at least suggestive to place the aster-yellows virus at one end of the scale and, at the other end, a virus that may eventually be shown to be transmissible only through vegetative means. Or, there is some reason to believe that a virus that is least specialized, *i.e.,* transmitted by various means and to a wide host range, may more truly represent one end of such a scale. As far as our own present conception of the grouping and order of classification of plant viruses is concerned, the main features are represented in the descriptive key presented.

SUMMARY

The need for a more complete and systematic description of plant viruses is discussed. The suggestion is again made that description should be based upon the characters of the virus itself rather than upon the disease which it causes.

The chief diagnostic features of a virus that are of value for descriptive purposes are believed to be: (1) Modes of transmission; (2) natural or differential hosts; (3) longevity *in vitro;* (4) thermal death point; (5) certain distinctive or specific symptoms.

Transmission by sucking insects and the identity of the vector concerned,

together with mechanical transmission by plant extract and transmission by grafting, constitute the main bases for differentiation.

By utilizing the above criteria in particular, it has been possible to draw up a descriptive key for plant viruses that places about 50 of the known forms in several fairly well-defined groups.

The suggestion is made that the various groups bear a certain natural relation to each other, and that a logical basis for classification may develop for more intensive studies of these relationships.

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A LEAF SPOT OF BENT GRASSES CAUSED BY HELMINTHOSPORIUM ERYTHROSPILUM, N. SP.

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HISTOLOGICAL REVIEW, DISTRIBUTION, HOST RANGE, ECONOMIC IMPORTANCE

In a comparative account (1, p. 684) of various graminicolous members of the genus Helminthosporium that was published in 1923, reference was made to the occurrence on redtop, *Agrostis alba* L., and on thin grass, *A. perennans* (Walt.) Tuckerm., of 2 apparently undescribed species of Helminthosporium having larger conidia than *H. dematioideum* Bub. & Wrob., a form now and then also found on these grasses. The 2 fungi in question had been observed frequently on withered leaves of the bent grasses collected on Long Island near Brooklyn, N. Y., as well as in southwestern Connecticut between Stamford and Norwalk at different times late in June and during July in both 1920 and 1921. As in the absence of distinctive lesions the withering had much the appearance of drought injury, it was not possible then to attribute to either of the fungi any definitely disease-producing rôle.

Beginning with August, 1921, observations on redtop and other bent grasses were continued in the District of Columbia and adjacent portions of Maryland and Virginia. A withering of foliage resembling that previously seen in New York and Connecticut, was in evidence here also, and mostly, again, without any intervention of well-marked lesions. Microscopic examination of the dead leaves rather regularly revealed the presence of conidiophores and conidia of the one of the two larger species of Helminthosporium that had been by far the more abundant on redtop in the region surrounding New York City. During July, 1923, this species occurred on redtop in such definite relation to dull reddish grey foliar lesions that its causal connection with the injury became entirely obvious. In a brief abstract (3) based on observations then made the conidia were described as straight, cylindrical, olivaceous, 1- to 9-times septate, and germinating by the production of lateral and oblique germ tubes from intermediate as well as end segments. The details given were thus sufficient to place the fungus in the series of forms having their ascigerous stages in the genus Pyrenophora, especially as the antithesis between the two main series of graminicolous species of Helminthosporium with respect to both the conidial and the sexual stages was pointed out in another abstract (2) that appeared at the same time. Mention was made of the production of numerous small sclerotia in culture as a feature distinguishing the fungus from the parasite

causing leaf spot of oats; and the smaller dimensions of its conidia were cited as distinguishing it from *H. siccans* Drechsl., likewise a member of the Pyrenophora series.

Once the appearance of the more distinctive type of foliar injury due to the parasite was known, it was not difficult to recognize the pathological effects of the fungus on redtop in stands near Washington, D. C., nearly every season following 1923. When occasion offered opportunity for observation elsewhere, the same injury in association with the same fungus was found in various localities in the Middle West; as, for example, near East Lansing, Mich., in July, 1927; near La Fayette, Ind., in August, 1928; near Madison, Wis., in July, 1931; and near Columbus, Ohio, in July, 1933. Apparently the parasite is widely distributed wherever redtop is grown in the more humid States of our northern and middle latitudes, its prevalence and abundance here in any locality and season depending much on local weather conditions. All in all it seems certainly to be one of the most serious parasites affecting redtop, not any of which, to be sure, are especially devastating in their action. The economic loss accruing from it is perhaps not very considerable. Yet the possibility is hardly to be overlooked that damage to pasture and forage grasses brought about by leaf diseases may be greater than, in the absence of any good means of estimating injury of such origin, is now generally supposed.

Besides the agriculturally very valuable redtop, the fungus under discussion is known to attack also seaside bent, *Agrostis palustris* Huds., (formerly cited as *A. maritima* Lam.), and colonial bent, *A. tenuis* Sibth. Colonial bent was seen attacked only feebly, the strain of this grass growing at Arlington Experiment Farm, Rosslyn, Va., in 1928, showing only a few scattered lesions (Fig. 1, F–H) during the prolonged wet period in September of that year. The remarkably heavy infection that took place on seaside bent at that time was referred to in an earlier paper (4, pp. 129, 130, 132). Several of the strains of creeping bent, *A. palustris* Huds., (previously cited as *A. stolonifera* L.), widely used for turf on golf courses, bore lesions that appeared very probably referable to the Helminthosporium common on redtop, though the abundance of the zonate-eyespot fungus, similarly encouraged by the wet weather, precluded a wholly certain identification. Even greater difficulty attended the attempted identification of reddish lesions found on velvet bent, *A. canina* L., owing to the narrowness of the leaves taken together with the abundance of zonate-eyespot lesions in all stages of development.

SYMPTOMS AND PATHOLOGICAL EFFECTS

That the parasite has so long escaped observation in spite of its widespread distribution on an important economic plant, is undoubtedly to be

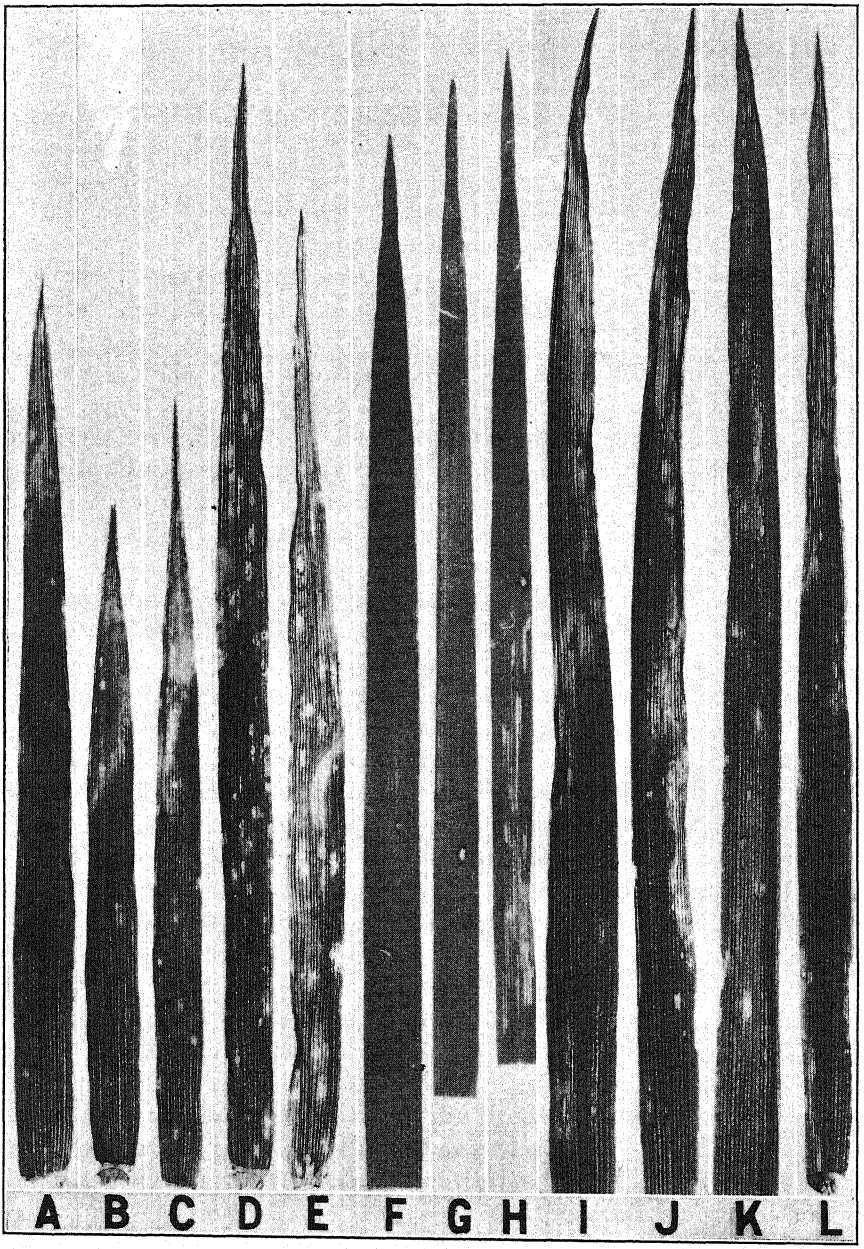


FIG. 1. A–E. Redtop leaves with lesions and general withering effects brought about by *Helminthosporium triseptatum;* photographed from material collected at Arlington Experiment Farm, Sept. 11, 1926. ×2. F–H. Leaves of colonial bent with single lesions due to invasion by *H. erythrospilum;* photographed from material collected at Arlington Experiment Farm, Sept. 26, 1928. ×2. I–L. Leaves of seaside bent collected at Arlington Experiment Farm, Sept. 28, 1928, showing definite lesions due to *H. erythrospilum* that had developed in rainy weather, and withering effects that soon appeared when drier conditions later intervened. ×2.

attributed in large part to the frequently deceptive appearance of the injury caused by it. In dry seasons and often even in moderately wet seasons the local discoloration associated with the inception and enlargement of separate invaded areas soon becomes obscured in the general withering of the leaf that follows apparently as a secondary effect of invasion. Consequently, affected redtop leaves frequently present a drought-stricken appearance, though the moisture available may be more than enough to keep healthy plants in full vegetative luxuriance.

During the 15 seasons in which my observations were continued, the essentially local character of the separate infections was never brought into relief more clearly than in the heavy attack of the fungus on seaside bent in September, 1928, to which reference has already been made. With the unusually moist and warm weather that came in the first 3 weeks of that month, infections multiplied on the luxuriant foliage, while at the same time the secondary withering effects were almost entirely avoided (Fig. 2, A–L). On most of the leaves were borne lesions from 5 to 25 in number, individually appearing often as straw-color spots, 0.5 to 0.8 mm. wide and 0.5 to 2.5 mm. long, surrounded by a reddish or brownish red border of variable width. Sometimes the straw-color center was very minute or entirely absent, and the spot then rather closely resembled the lesions that had become familiar on redtop under more ordinary weather conditions. Collections made during the prolonged intervals of precipitation showed a large proportion of the discolored spots surrounded individually by a water-soaked belt of variable width, representing undoubtedly an increment of newly occupied tissue. These zonal increments are illustrated fairly well in figure 2, D–L, showing affected leaves photographed immediately on collection after 16 hours of drizzling rain. The more extensive zonate lesions (Fig. 2, K, L) thus sometimes came to present a pattern somewhat suggestive of lesions caused by *Helminthosporium giganteum* on its more favorable hosts, among which, however, the strain of seaside bent in question is not to be reckoned. The differences between the simple eyespot lesions on this grass caused by *H. giganteum* and those due to the fungus under discussion have been previously set forth (4, p. 132), and become readily evident on a comparison of the pertinent illustrations of the earlier paper (4, Pl. 1, J–S) with those included in figure 2 of the present contribution. Rather curiously, the lesions produced by *H. giganteum* on seaside bent resemble those produced by the leaf-spot fungus on the same host (Fig. 2, A–L) much less than they resemble those produced by the latter parasite on the specifically different colonial bent (Fig. 1, F–H).

Weather conditions that permit the development of leafspot lesions as definite and numerous as those present on the leaves shown in figure 2,

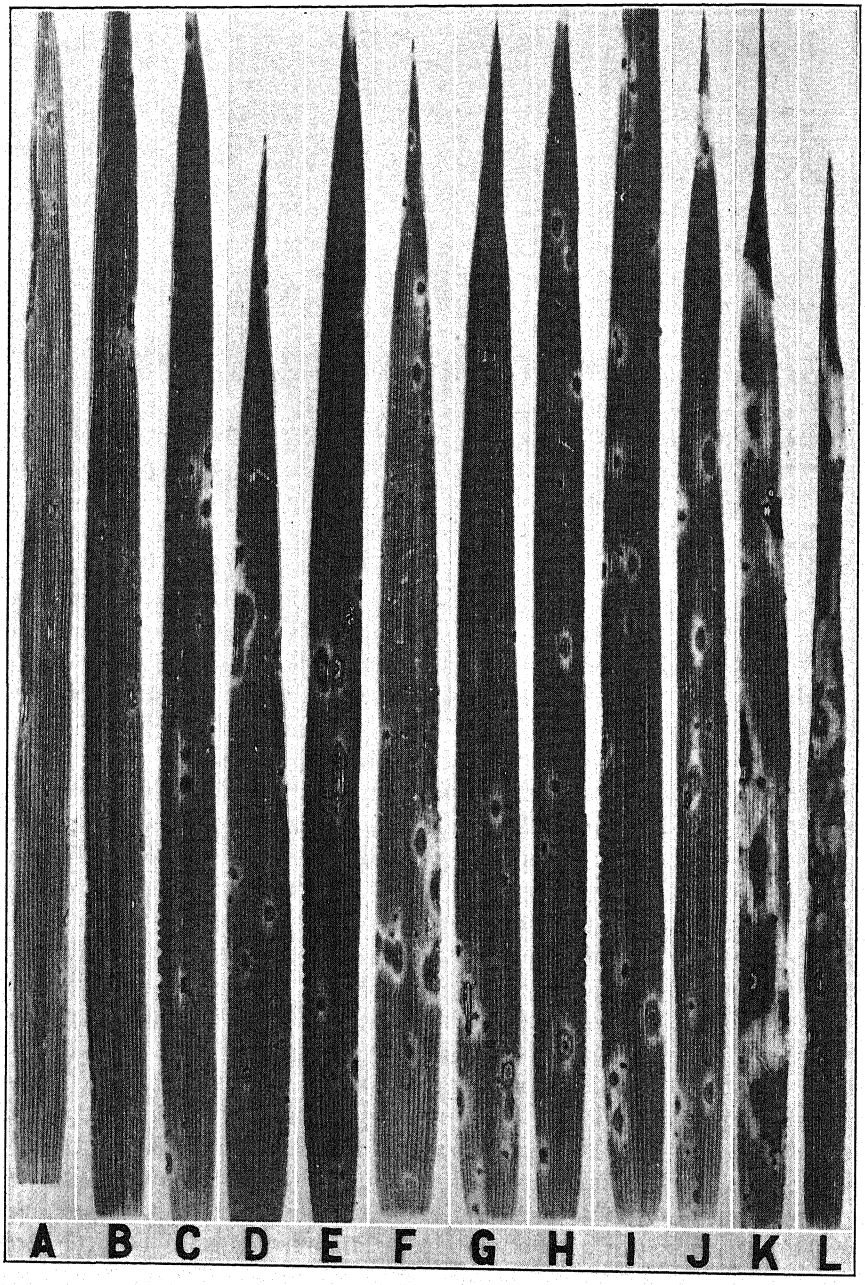


FIG. 2. A–L. Leaves of seaside bent arranged approximately in the order of increasing severity of attack by *Helminthosporium erythrospilum*, collected at Arlington Experiment Farm, Sept. 19, 1928, after 16 hours of misty rain. The light zones surrounding many of the spots represent water-soaked areas newly invaded by the fungus. Symptoms of withering are absent except in K, L. ×2.

are not wont to occur frequently, at least in the regions in which my observations were made. When such conditions come to an end, withering of the more seriously affected leaves soon intervenes; and with the consequent obliteration, in large part, of local discoloration, much of the distinctiveness of the earlier pathological picture is lost. Thus several days after the rains prevailing when the material shown in figure 2 was collected had come to an end, the diseased foliage in the same plot of seaside bent from which the collections were made showed the leafspot discoloration considerably faded, and the blades themselves in process of withering from tip toward base (Fig. 1, I–L).

COMPARISON WITH A REDTOP LEAF SPOT DUE TO HELMINTHOSPORIUM TRISEPTATUM

In the region surrounding New York City, as has been noted previously, *Helminthosporium dematioideum* was occasionally found during the season of 1920 on withered foliage of redtop and of thin grass. However, no well marked foliar lesions could at any time be connected with the presence of this fungus, even though it was encountered on redtop in small quantity from time to time in later seasons near the District of Columbia. Manifestly not much pathogenic activity is to be attributed to *H. dematioideum* as far as bent grasses are concerned. Indeed it would not be difficult to deny any injurious action on these grasses whatsoever, except for the fact that the foliage of sweet vernal grass, *Anthoxanthum odoratum* L., is almost equally free of local discoloration up to the time it begins to shrivel and put forth conidial apparatus of *H. dematioideum* in such quantity and with such regularity as to indicate a parasitic relationship.

In several of the last 14 seasons *Helmnthosporium triseptatum* Drechsl., a species first described (1, pp. 685–686) as occurring on velvet grass, *Holcus lanatus* L., has been found also on fading leaves of redtop as well as of timothy, *Phleum pratense* L., in Virginia and Maryland near Washington, D. C. In some especially favorable redtop material this fungus could be made out as the cause of rather minute foliar lesions consisting of a pale central area surrounded by a salmon-color zone (Fig. 1, A–E). Horsfall (5) found a fungus he identified provisionally as *H. triseptatum* to be the cause of a leafspot on redtop and on orchard grass, *Dactylis glomerata* L., in the State of New York. The lesions on redtop described by Horsfall would seem to have been somewhat darker than those caused by *H. triseptatum* on this grass in Virginia. In New York, moreover, the spots were described as becoming rapidly elongated longitudinally, forming streaks through which the death of the leaf is brought about; whereas, in Virginia, such death would seem to be introduced by general withering of the organ as a whole. These differences very probably find their explanation in the

rather considerable difference in environmental conditions obtaining between the two regions. Whatever the explanation may be, however, the individual lesions due to *H. triseptatum* in Virginia and Maryland, are usually distinguishable from those due to the fungus herein described, in their smaller dimensions and paler coloration. Of the two parasites, the former appears to be by far the less destructive one, at least as far as the bent grasses are concerned.

MORPHOLOGY OF THE CAUSAL FUNGUS ON THE NATURAL SUBSTRATA

The fungus responsible for the more serious, russet leaf spot is regularly to be found on the more extensive tracts of tissue killed by it (Fig. 3, A, a–c). Its conidiophores first generally appear in the centers of the larger discolored areas. Later they arise, somewhat more sparsely, from areas not included in the originally infected lesions. In either position they emerge from the host epidermis singly (Fig. 3, B, a–c, f) or in pairs (Fig. 3, B, d, e), rarely in larger groups. Emergence ordinarily takes place between epidermal cells; when stomata are utilized (Fig. 3, B, d, e) it is apparently more through accident than from physical constraint such as is evidently exerted by the structurally much sturdier epidermis of maize, *Zea mays* L., on the conidiophores of *H. turcicum* Pass. In dimensions, coloration, and septation, as in the presence of geniculate bends marking the points at which successive conidia were formed, the conidiophores of the present fungus show little to distinguish them from the homologous structures of congeneric parasites.

The cylindrical shape of the conidia (Fig. 3, C, a–z, aa–ad) and their indiscriminate mode of germination (Fig. 3, D, a–g) refers the fungus to the series of forms in Helminthosporium that have their ascigerous connections, wherever these are known, in the genus Pyrenophora—the series essentially corresponding, therefore, to the subgenus Cylindro-Helminthosporium of Nisikado (7, 8) and to the genus Drechslera of Ito (6). Though the conidia are by no means small in comparison with fungus spores generally, they are yet so much smaller than those of some of the larger members of the Pyrenophora series—*H. bromi* Died., *H. tritici-repentis* Died., and *H. teres* Sacc.—that the possibility of specific identity is convincingly excluded through the disparity in conidial size alone. Through their greater dimensions can be excluded from consideration also *H. tritici-vulgaris*, a species parasitic on wheat in Japan (7, 8), and *H. giganteum*, which, in view of its peculiarities in germination and in Hormodendron-like aerial proliferation, can only very doubtfully be considered a member of the series.

However, the differences in conidial dimensions are less pronounced when comparison is made with the less robust members of the Pyrenophora series. Among these members are to be reckoned, besides the barley-stripe

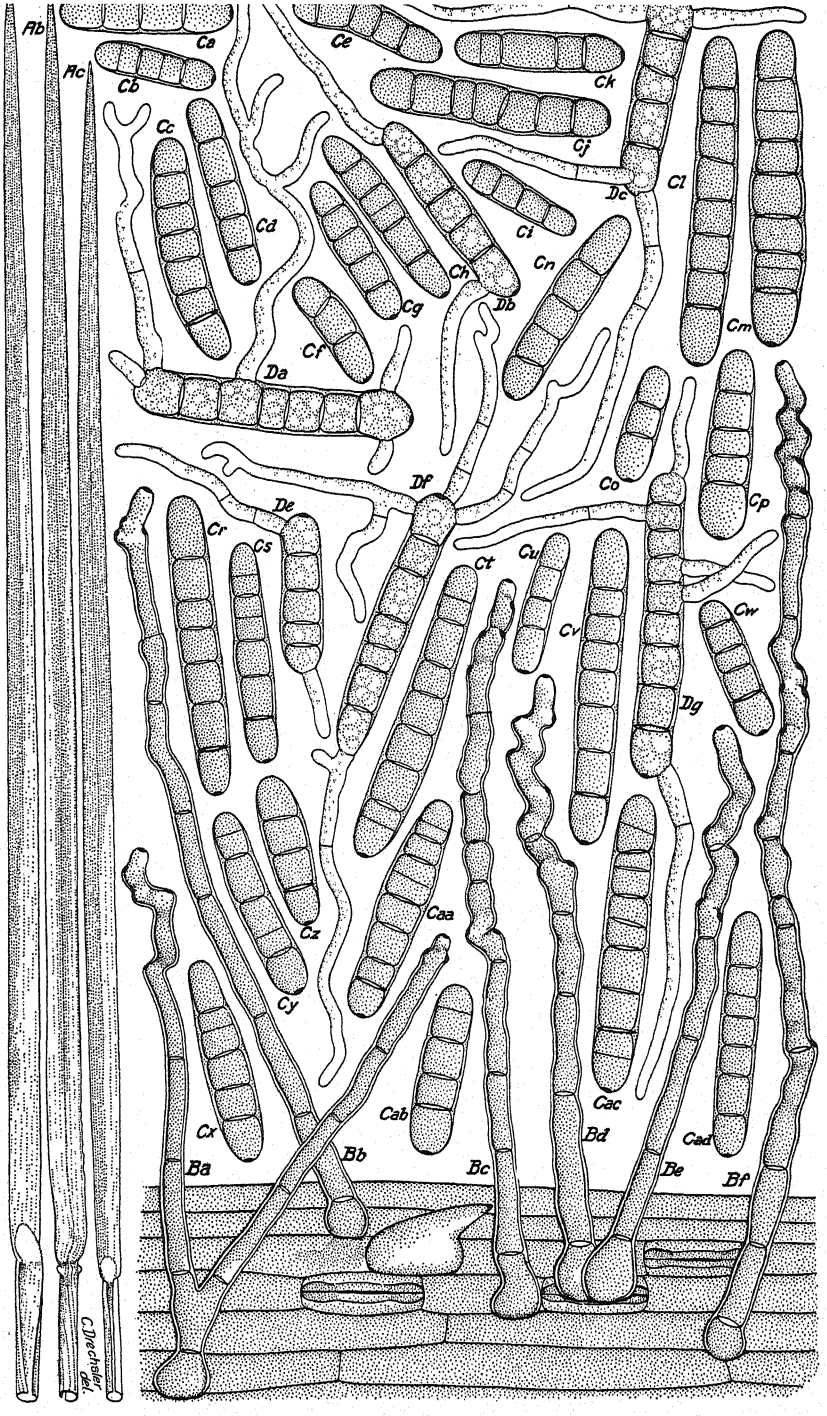


FIG. 3. *Helminthosporium erythrospilum* on redtop, drawn from material collected at Arlington Experiment Farm, July 16, 1923. A, a–c. Affected leaves of redtop, the more heavily stippled areas representing withered regions over parts of which conidiophores of the fungus are borne. ×1. B, a–f. Conidiophores emerging in various relationships to epidermal cells. ×500. C, a–z, aa–ad. Conidia from redtop leaves showing variations in size, shape, and septation. ×500. D, a–g. Conidia in early stages of

TABLE 1.—*Lengths of 200 conidia produced by several species of Helminthosporium on their respective grass hosts in nature, distributed in classes with ranges of 10μ*

Fungus	21 to 30μ	31 to 40μ	41 to 50μ	51 to 60μ	61 to 70μ	71 to 80μ	81 to 90μ	91 to 100μ	101 to 110μ	111 to 120μ	121 to 130μ	131 to 140μ	141 to 150μ	151 to 160μ	161 to 170μ	Average length
H. erythrospilum (a)	2	15	29	29	48	40	24	12	1							64.3μ
H. erythrospilum (b)	1	16	18	43	42	48	23	9								65.2μ
H. dictyoides	2	14	20	23	24	29	38	30	13	4	2				1	74.5μ
H. siccans		4	13	19	20	49	41	26	16	5	3	3		1		80.1μ
H. gramineum		11	21	25	44	42	38	15	2	1		1				69.8μ
H. avenae		1	3	11	17	44	42	36	17	19	7	2	1			87.6μ

TABLE 2.—*Widths of 200 conidia produced by several species of Helminthosporium on their respective grass hosts in nature, distributed in classes with ranges of 1μ*

Fungus	8μ	9μ	10μ	11μ	12μ	13μ	14μ	15μ	16μ	17μ	18μ	19μ	20μ	21μ	22μ	24μ	Average width
H. erythrospilum (a)	2	15	15	40	68	35	20	5									11.8μ
H. erythrospilum (b)	3	4	6	16	45	64	35	21	6								12.8μ
H. dictyoides				1		3	21	38	58	49	26	3	1				16.1μ
H. siccans				1	1	4	7	22	39	66	41	15	4				16.8μ
H. gramineum	1			1	4	4	11	19	21	34	41	38	14	7	4	1	17.4μ
H. avenae					1	2	10	23	44	57	50	12	1				16.7μ

fungus, *Helminthosporium gramineum,* such parasites as *H. dictyoides* Drechsl., the cause of a fairly serious net blotch of meadow fescue, *Festuca elatior* L., and *H. siccans* Drechsl., the cause of a widespread leaf spot affecting perennial rye grass, *Lolium perenne* L., and Italian rye grass, *L. multiflorum* Lam. Like the bent grass parasite, *H. dictyoides* and *H. siccans* as well as *H. gramineum* produce conidial apparatus during June and the earlier weeks of July. Meadow fescue and the rye grasses are often found in fields intermingled with redtop, so that circumstances of local propinquity are present to encourage a supposition that the parasites on the grasses might belong to a single species. Yet, on closer examination, differences in conidial morphology become evident entailing separation of the parasites into species along lines corresponding to the generic separation of the host plants.

These differences are brought into relief in tables 1, 2 and 3, in which are shown the distribution in appropriate classes and the computed averages of measurements of the lengths and the diameters of 200 conidia taken from field material of the several members of the Pyrenophora series of most immediate concern, together with the corresponding distribution and averages of the numbers of septa in the conidia measured. The two separate sets of measurements of the bent-grass parasite are given under the binomial *Helminthosporium erythrospilum,* one set (*a*) being derived from spores taken from infected leaves of redtop collected near La Fayette, Ind., Aug. 25, 1928, the other set (*b*) from conidia taken from leaves of seaside bent collected at Arlington Experiment Farm, Sept. 19, 1928. The measurements of *H. dictyoides* were made on spores scraped from diseased leaves of meadow fescue gathered at Arlington Experiment Farm, June 7, 1934; and affected foliage of perennial rye grass gathered also at Arlington Experiment Farm, June 7, 1934, was used in making the measurements of *H. siccans.* Barley affected with stripe, originating from Virginia early

TABLE 3.—*Numbers of septa in 200 conidia produced by several species of Helminthosporium on their respective grass hosts in nature, distributed in classes*

Fungus	1	2	3	4	5	6	7	8	9	10	11	Average septation
H. erythrospilum (*a*)		7	17	40	24	71	27	11	2	1		5.4
H. erythrospilum (*b*)		6	19	37	40	50	25	19	3	1		5.4
H. dictyoides	5	23	20	61	41	35	10	3	1		1	4.4
H. siccans		13	13	50	64	35	14	6	4	1		4.9
H. gramineum	1	24	42	66	40	21	6					4.0
H. avenae	2	4	9	72	42	54	11	3	2		1	4.9

in June, 1934, was used in making the measurements of *H. gramineum*. Though *H. avenae* is hardly to be considered among the smaller members of the series, measurements of that species are included for comparison; they were made on conidia taken from leaves of oats, *Avena sativa* L., severely affected with leaf stripe collected at Arlington Experiment Farm, June 26, 1934.

The data given in tables 1–3 show in a somewhat quantitative manner relations that are readily perceptible when material of the several species are examined side by side. Though the conidia of the bent-grass parasite are appreciably shorter than those of *H. gramineum* and of *H. dictyoides*, the inferiority in length, especially in comparison to the stripe fungus, is not at all pronounced. On the other hand, the inferiority in width of conidium relative to any of the other four species is striking and undoubtedly significant, since this dimension is much less given to wide variation than is the length. Again, although the conidia of the bent-grass parasite are shorter than those of the other species represented in table 1–3, they are, nevertheless, on the whole more abundantly septate. As a result, the conidial segments are not only narrower than those of the other species but also shorter, and that in a measure more than proportional to the smaller length of the conidium.

From the accounts of Nisikado (7, 8) it is evident that the conidia of *Helminthosporium arundinis* Lév., a parasite causing leaf spot of *Arundo donax* L., approach those of the bent-grass fungus more closely in length, width, and frequency of septation, than do the conidia of either *H. dictyoides, H. siccans, H. gramineum* or *H. avenae*. With respect to shape, however, the correspondence would seem not especially close. The end cells in the conidia of *H. arundinis* are described as comparatively narrow; and this condition entails, as is apparent in Nisikado's figures, a more pronounced tapering of the spore in its apical and basal portions than is typical of the bent-grass parasite, whose conidia are as a rule abruptly rounded off at both ends. Moreover, the protruding position of the hilum set forth among the distinctive features of *H. arundinis* offers a marked contrast to the inclusion of the homologous scar within the basal contour of the conidium in the parasite on redtop and seaside bent.

Cultural Characters of the Parasite

The isolation of *Helminthosporium erythrospilum* offers no unusual difficulties. When vegetatively vigorous leaves with newly established lesions are available, the invaded portions of tissue may be excised, and after surface sterilization and repeated washing in sterile water, planted on some suitable medium like maize-meal agar. From most plantings thus made, mycelium of the parasite will grow out, free from contamination with

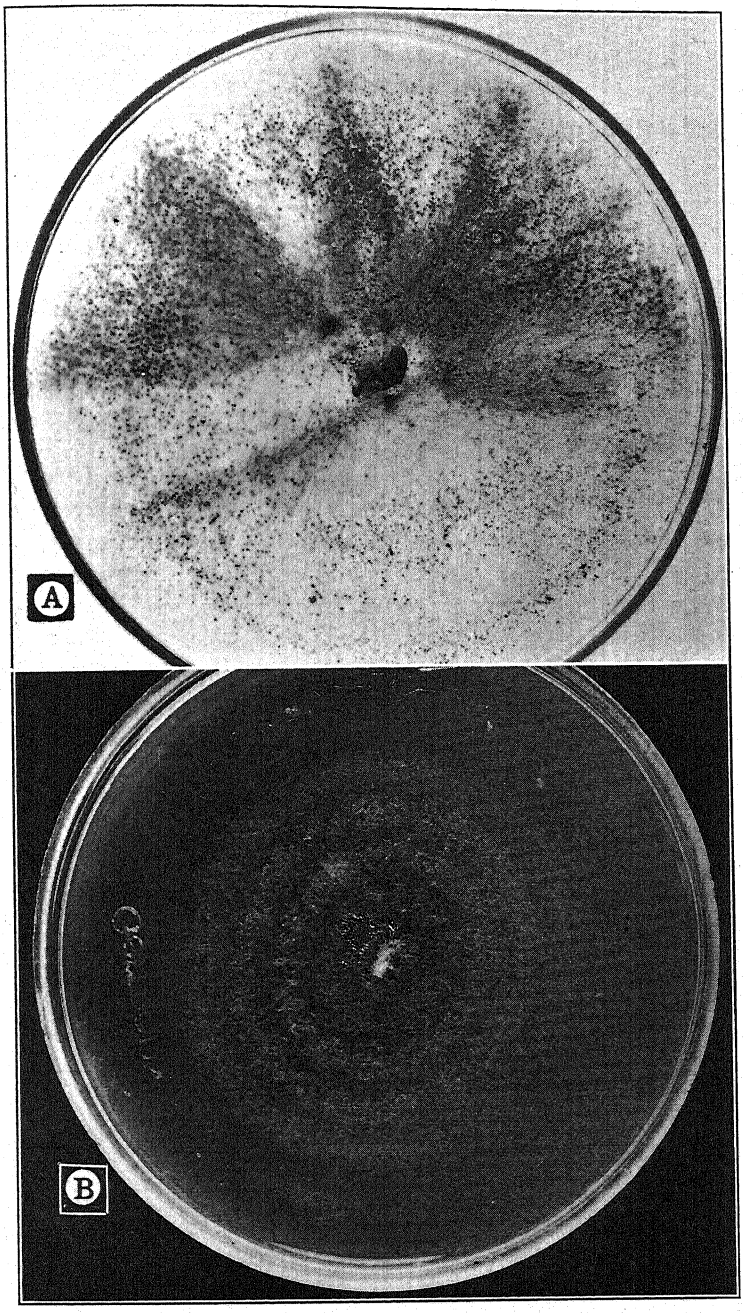


FIG. 4. Maizemeal-agar plate cultures of *Helminthosporium erythrospilum*. ×1. A. Culture incubated for 30 days at approximately 15° C., showing numerous submerged sclerotia. B. Culture after 6 days growth at a temperature of approximately 30° C., showing character of aerial mycelium.

other organisms. When withered leaves bearing conidial apparatus need to be used, plain agar dilution plates may advantageously be employed to make possible the removal of single germinating conidia to new cultures.

The fungus once isolated grows well on artificial media, showing a fairly rapid rate of linear extension. On maize-meal-agar plates at 30° C., it produces in several days a light grey or steel grey aerial mycelium (Fig. 4, B) rather strongly resembling that of *Helminthosporium siccans* (Fig. 5, B), though ordinarily somewhat less copious and often more given to zonation. Accordingly, like also the latter species, it is easily distinguished from *H. dictyoides,* which, in maize-meal-agar plate cultures, develops a more abundant, almost white, somewhat columnar or tufted mycelium (Fig. 5, A).

What is perhaps the most distinctive character of *Helminthosporium erythrospilum* on artificial media comes to light when cultures of it are kept at rather low temperatures (10 to 20° C.) for 2 weeks or longer. Numerous minute dark sclerotia scarcely exceeding 0.1 mm. in diameter make their appearance scattered through the substratum. A dense arrangement in the presence of abundant food materials (Fig. 4, A) yields to a more diffuse distribution in a less nourishing substratum (Fig. 6, B). Apparently, these sclerotia are of the same sort as the much larger but otherwise similar bodies that are produced in cultures of *H. bromi* and that evidently represent immature perithecia. Sclerotia much like those of *H. bromi,* and hence greatly exceeding those of the bent-grass parasite in size, are produced often on maize-meal-agar cultures of the Pyrenophora occurring fairly abundantly on old oat straw in Virginia, Maryland, and Delaware, during May and early June (Fig. 6, A).

The obvious disparity in size between the sclerotia of *Helminthosporium erythrospilum* and the sclerotia of the Pyrenophora on oats is of moment, especially as the two fungi, unlike most other members of the same natural series, sporulate fairly readily and fairly abundantly in pure culture. When, for example, maize-meal-agar plate cultures of the bent-grass parasite are kept for a period of 50 days at a temperature of approximately 18° C. and protected from evaporation, conidiophores (Fig. 7, A–F) abundantly laden with conidia (Fig. 7, A) are produced. In coloration, septation, and dimensions these conidia (Fig. 7, G, a–n) show little dissimilarity from those produced under natural conditions. Nor are the conidiophores essentially different from those produced in nature, though as might be expected from analogy with species of Helminthosporium referable to the Cochliobolus series, which in general sporulate very freely on artificial substrata, the prolonged conditions suitable for development permit these structures to attain greater length and to bear a proportionately larger number of conidia.

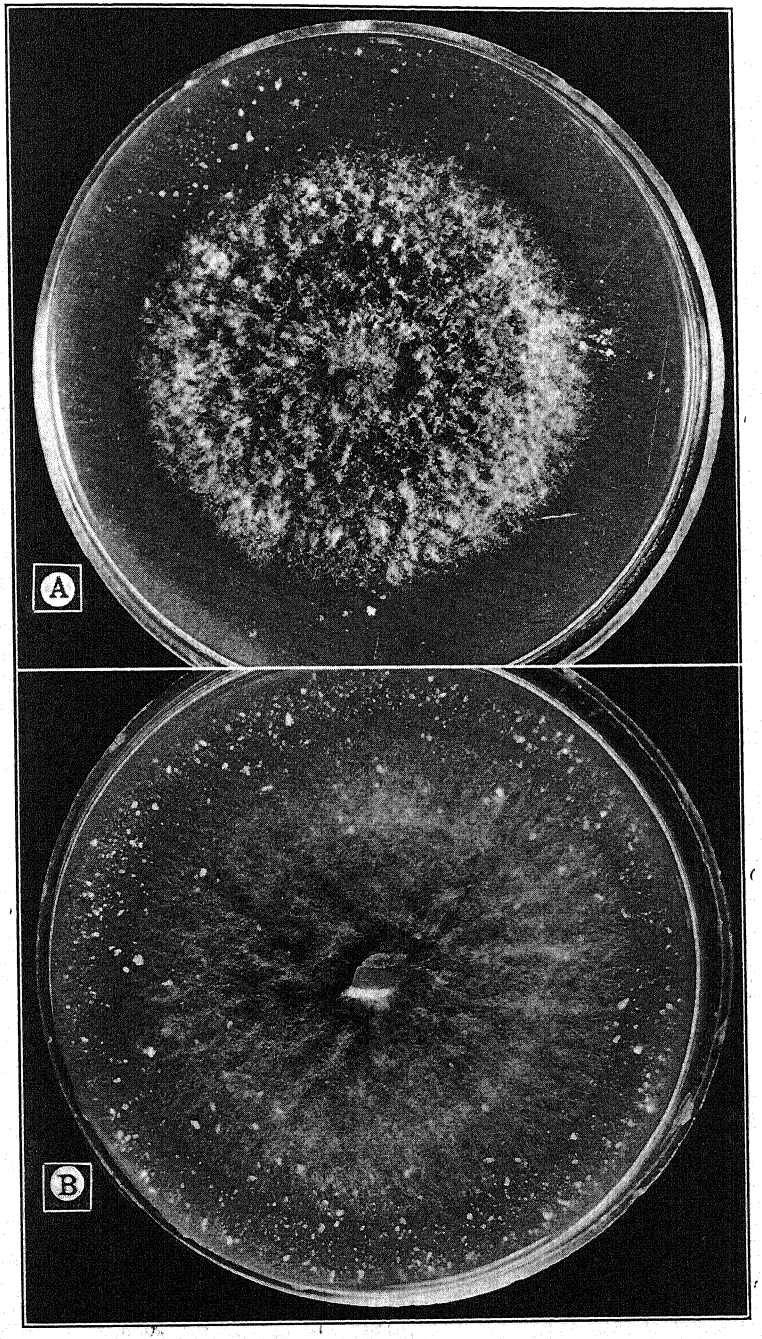


FIG. 5. Maizemeal-agar plate cultures of 2 species of Helminthosporium comparable to *H. erythrospilum* in conidial dimensions, showing appearance of aerial mycelia after 6 days' growth at a temperature of 30° C. ×1. A. *H. dictyoides*. B. *H. siccans*.

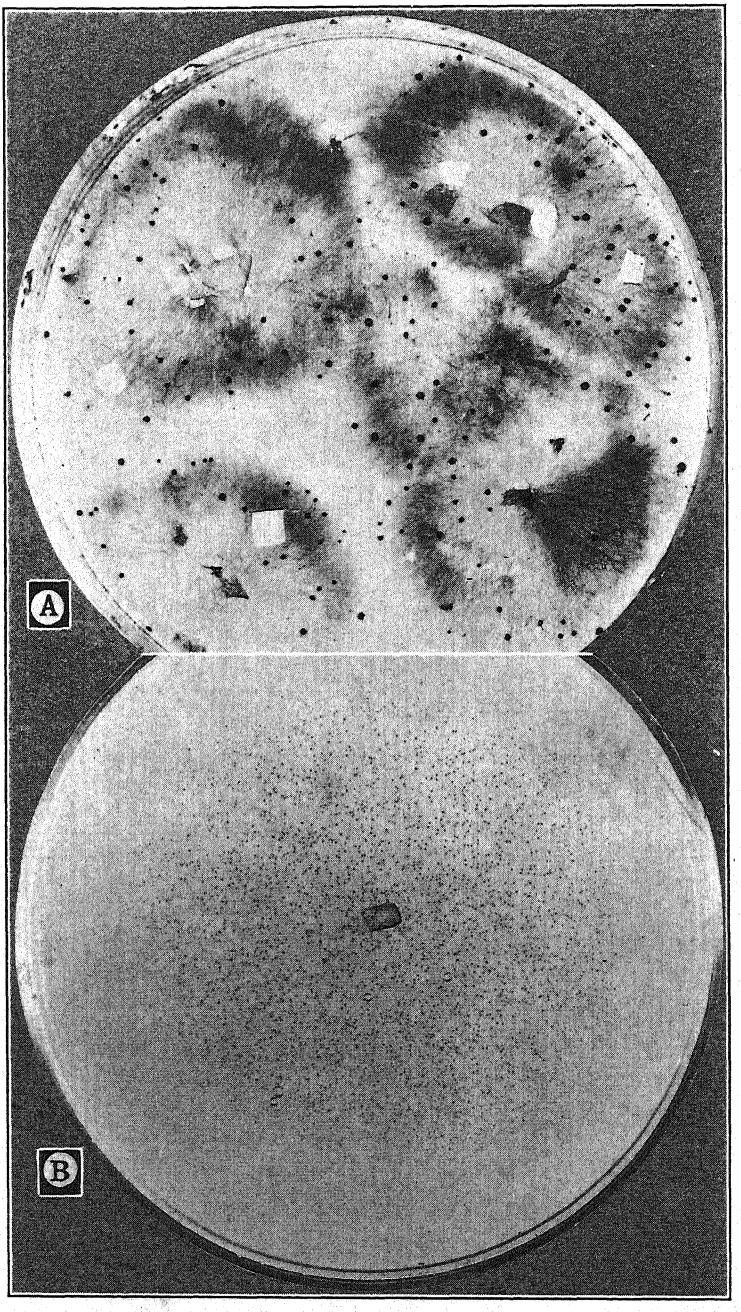


FIG. 6. A. Maizemeal-agar plate culture of the Pyrenophora sp. with ascospores having 5 transverse septa, that occurs abundantly on overwintered oat straw in Virginia, Maryland and Delaware; showing the relatively large sclerotia characteristic of the fungus. ×1. B. Maizemeal-decoction-agar plate culture of *Helminthosporium erythrospilum* after incubation for 30 days at a temperature of about 15° C.; the medium being poor in nutrients compared with that used in the culture shown in Fig. 4 A, has permitted the development of correspondingly fewer sclerotia. ×1.

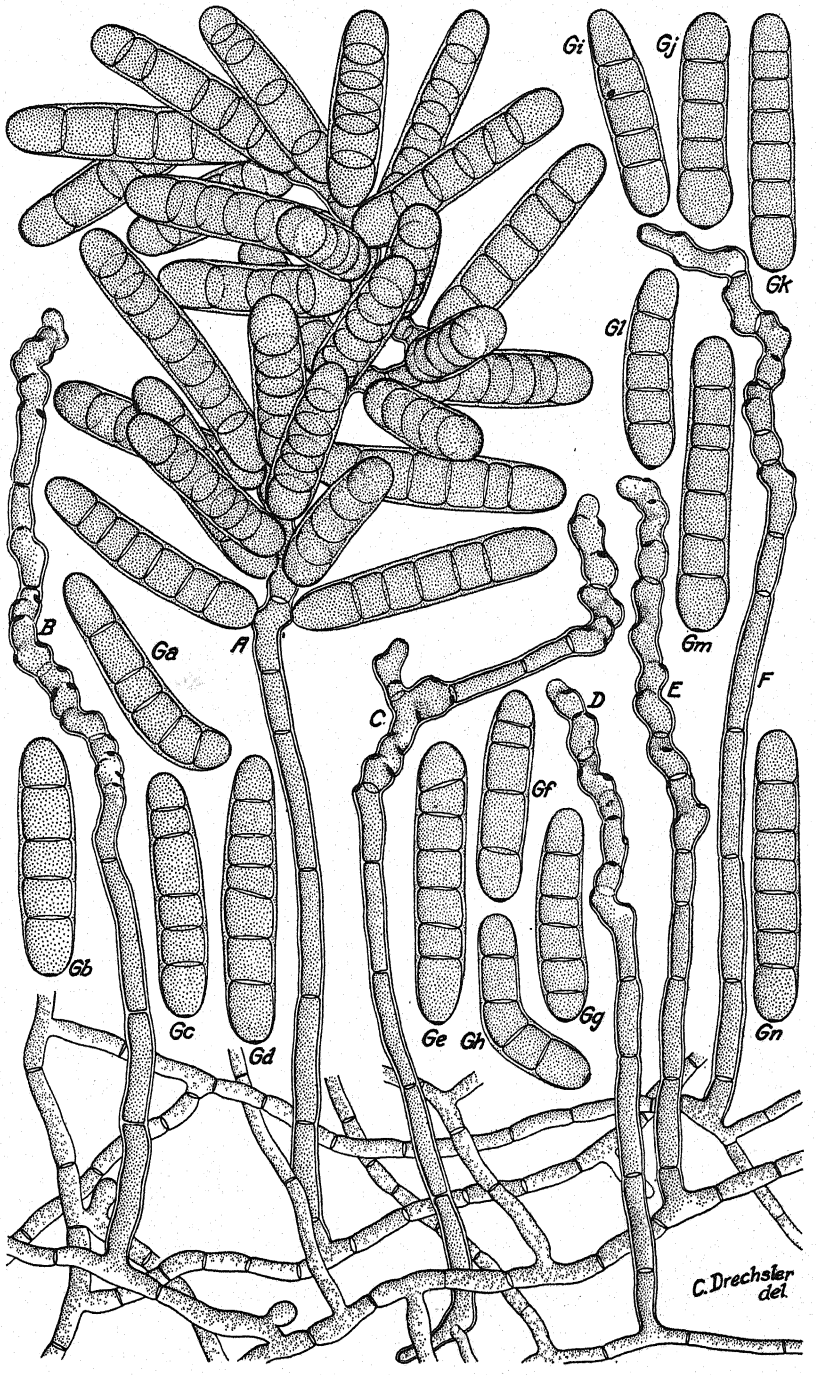


FIG. 7. *Helminthosporium erythospilum* in pure culture on a maizemeal-agar plate culture after incubation for 50 days at a temperature of approximately 18° C. ×500. A. Conidiophore with attached conidia. B–F. Denuded conidiophores. G, a–n. Conidia.

DESCRIPTION OF THE PARASITE AS A NEW SPECIES

As the parasite under consideration differs morphologically from all of the several congeneric forms assignable to the same series, it deserves description as a new species. The specific name, meaning "red spot," proposed for it is intended to be broadly descriptive of the coloration often present in leaf areas occupied by it, without, however, implying that such coloration constitutes an invariable diagnostic character of the fungus.

Helminthosporium erythrospilum, n. sp.

Sparsum; hyphis fertilibus erectis, fuligineis, simplicibus vel rarius furcatis, nodulosis, hinc illinc geniculatis, 75–275 μ longis, 6–9 μ crassis, 1–10 septatis; conidiis flavidis vel dilute olivaceis, typice rectis sed interdum nonnihil curvatis, cylindraceis, utrimque abrupte rotundatis, 2–10 septatis, ad septa non constrictis vel minute constrictis, saepius 25–105 × 8–16 μ, e cellulis pluribus germinantibus, hilo in membrana incluso.

Habitat in foliis vivis vel languidis Agrostidis albae, Agrostidis palustris et Agrostidis tenuis in Amer. bor.

Conidiophores erect, brown or fuliginous, simple or occasionally somewhat branched, emerging singly or less often in pairs or larger groups mostly from between epidermal cells but sometimes from stomata, measuring mostly 75 to 275 μ in length by 6 to 9 μ in diameter, producing the first conidium usually 75 to 125 μ from the base and later conidia at variable successive intervals marked by geniculations; containing 1 to 10 septa that delimit segments mostly 15 to 35 μ long.

Conidia when mature usually distinctly yellowish, sometimes even light olivaceous; typically straight, rarely somewhat curved, mostly nearly cylindrical, the ends rather abruptly rounded off, containing 2 to 10 septa that are usually not marked by constrictions, but at times may be associated with perceptible constrictions; measuring usually 25 to 105 μ (average 65 μ) in length by 8 to 16 μ (average 12.3 μ) in diameter; individually including the nonprotruding hilum within the basal contour; germinating rather indiscriminately from any or all cells, but usually more abundantly from the thin-walled basal and apical cells than from the intermediate cells.

Attacking leaves of redtop, *Agrostis alba,* and seaside bent, *A. palustris,* on which it produces reddish or yellowish red or russet brown spots with bleached centers, the local discoloration later becoming more or less obliterated with the withering of the individual leaves from tip toward base. Causing also localized lesions on the leaves of colonial bent, *A. tenuis.* In Connecticut, New York, District of Columbia, Maryland, Virginia, Ohio, Indiana, Michigan and Wisconsin.

SUMMARY

A leaf spot has been found to occur widely on redtop in some Eastern and Middle-Western States. Under wet conditions it gives rise to localized

lesions, these being marked individually by russet discoloration surrounding a relatively small, paler, central region. Local discoloration is less evident in dry weather, when infected leaves often wither in a manner suggestive of drought injury. The same disease occurs in severe form also on seaside bent. Lesions unaccompanied by withering effects have been observed on colonial bent.

Comparison is made between the disease and a much less injurious leaf spot attributable to *Helminthosporium triseptatum.*

The disease is caused by a species of Helminthosporium producing conidia typically straight cylindrical, rounded abruptly at both ends, distinctly yellowish in coloration, 25 to 105 μ long by 8 to 16 μ wide, germinating by the production of germ-tubes from any or all segments. The fungus is, therefore, referable to the series having ascigerous connections in Pyrenophora. It is described as a new species, *H. erythrospilum,* being distinguished from various other members of that series—*H. gramineum, H. dictyoides, H. siccans* and *H. avenae*—by the smaller diameter and closer septation of its conidia; from *H. arundinis* by its conidia being abruptly rounded at both ends, and by the position of the hilum within the contour of the basal segment. In pure culture on artificial media it often gives rise to submerged sclerotia that are more numerous and much smaller than those produced under similar conditions by *H. bromi;* or than those produced in pure culture by the Pyrenophora having ascospores with typically 5 transverse septa that occurs abundantly on overwintered oat straw in the Middle Atlantic States.

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THE USE OF THE QUINHYDRONE AND ANTIMONY ELECTRODES FOR DETERMINING THE pH OF SOLID CULTURE MEDIA[1]

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(Accepted for publication July 27, 1934)

In some studies conducted by the writer on the relation of the hydrogen-ion concentration of culture media to the growth of certain fungi, it became necessary to determine accurately the pH of solid culture media. The accuracy of colorimetric readings made on this type of media is so often questionable, due to the turbidity of the medium and the other errors to which this method is subject, that an effort was made to adapt the electrometric method.

The hydrogen electrode can not be used in solid media unless the buffer capacity of the particular medium is sufficient so that it can be diluted to the point where it will not solidify without altering the hydrogen-ion concentration. Lisse, Jensen, and Tittsler (8) found that Bacto-nutrient agar is sufficiently buffered to permit a 7-fold dilution without appreciably changing the pH. At this dilution, the medium remained liquid, and they were able to use the hydrogen electrode. The same authors report satisfactory results with the quinhydrone electrode in diluted Bacto-nutrient agar, and they prefer this electrode to the hydrogen electrode because it is much simpler to use.

Biilmann and Lund (5) and Biilmann (3) have shown that the quinhydrone electrode is sufficiently accurate to justify its replacing the hydrogen electrode in many instances. Later, Biilman, Klit, and Swaetichin (4) reported that the quinhydrone electrode gave correct pH values in phosphate mixtures up to pH 7.73; above this point the values were slightly too low, but they were fairly accurate even at pH 8.04.

The quinhydrone electrode was used by Watson (9) to follow the changes in pH in Swiss cheese during the process of making. The cheese and quinhydrone were mixed together in a mortar, packed in a glass tube, and then the electrode was inserted into the mixture.

Barnes and Simon (1) found that the antimony electrode could be used in determining the reaction of soils where the quinhydrone electrode could not be used because of the presence of considerable manganese. They worked out 3 equations to be used in changing the voltage obtained with the antimony electrode to pH units.

[1] Published with the approval of the Director of the Ohio Agricultural Experiment Station, Wooster, Ohio.

APPARATUS AND MATERIALS

A Leeds and Northrup Type-K potentiometer, Type-R galvanometer, Eppley standard cell, and storage batteries constituted the equipment for measuring the voltages. The Leeds and Northrup quinhydrone pH indicator No. 7654 was substituted for the apparatus mentioned above in some of the later determinations and proved to be quite satisfactory. The quinhydrone electrodes were made by fusing about 4 cm. of 21-guage platinum wire into the end of a piece of glass tubing. The protruding end of the wire was shaped into a coil. A saturated calomel half cell served as a reference electrode and a 2 per cent agar bridge, saturated with KCl served as a junction.

The antimony electrode was of the type described by Barnes and Simon (1) and consisted of an antimony rod cast from C.P. antimony soldered at one end to a copper wire. De Khotinski cement was used to seal the electrode into a glass tube so that about one inch of the antimony rod projected from one end of the glass tube and the copper wire extended through the opposite end.

EXPERIMENTAL

Bacto-nutrient, Bacto-corn-meal, Bacto-Lima bean, Bacto-bean pod, and Bacto-prune agars were made up from the dehydrated preparations of the Difco Laboratories, Detroit, Michigan, according to the manufacturer's recommendations. Potato-dextrose agar was included and was prepared according to the usual laboratory procedure. After autoclaving, a plate was poured from each lot of media, and then portions of each medium were diluted with distilled water 3, 5, 7 and 10 times and the diluted portions poured into Petri plates. Prune agar was the only one that solidified when diluted 5 times. All the others were semi-liquid at this dilution and all were liquid, or extremely soft, at dilutions of 7 and 10 times. The pH of the distilled water used was $5.9 \pm .2$.

The hydrogen-ion concentration measurements were made on the liquid samples by mixing quinhydrone with a small portion of the medium in a vial and then using the quinhydrone electrode. The reading was taken as soon after adding quinhydrone as possible. Readings were obtained on the samples that solidified by using the following procedure: A small amount of quinhydrone was sifted on an area, about 1 inch in diameter, of the surface of the medium. Then the quinhydrone and agar were mixed together by using a spatula. The electrode and free end of the KCl-agar bridge were placed immediately in the mixture of agar and quinhydrone, and the voltage reading taken.

Voltages were converted to pH by use of the standard conversion tables furnished by Leeds and Northrup. The laboratory temperature during

all of the work was approximately 25° C. The pH determinations on the various dilutions of 2 lots of the different media prepared at 2 different times are given in table 1.

TABLE 1.—*Effect of dilution on the pH of solid culture media, determined by the quinhydrone electrode*

Medium	Dilution	pH		Medium	Dilution	pH	
		Lot 1	Lot 2			Lot 1	Lot 2
Nutrient	None	7.33	7.32	Prune	None	6.27	6.16
	1:3	7.31	7.16		1:3	6.23	6.07
	1:5	7.21	7.06		1:5	6.20	6.04
	1:7	7.09	6.99		1:7	6.18	6.04
	1:10	6.96	6.92		1:10	6.21	6.02
Bean-pod	None	5.72	5.73	Lima bean	None	6.16	6.25
	1:3	5.85	5.77		1:3	6.32	6.28
	1:5	5.85	5.77		1:5	6.33	6.28
	1:7	5.94	5.77		1:7	6.36	6.27
	1:10	5.94	5.74		1:10	6.42	6.34
Corn meal	None	6.06	5.82	Potato	None	5.67	5.64
	1:3	6.16	5.85	dextrose	1:3	5.80	5.85
	1:5	6.18	5.97		1:5	5.86	5.88
	1:7	6.33	5.99		1:7	5.91	5.91
	1:10	6.67	6.24		1:10	5.94	5.95

Potato-dextrose agar was adjusted by the addition of H_3PO_4, mono-, di-, and tri-sodium phosphates after sterilization and before solidification so that a series of agars ranging from a very acid reaction to well above neutrality was obtained. The pH of each was determined colorimetrically by using a comparator and following the methods of Clark and Lubs (6). A small sample of each agar was mixed with quinhydrone in a mortar and the pH determined with the quinhydrone electrode as indicated above. Also, the quinhydrone was mixed with the agar, which had solidified in a Petri dish, by lowering and raising the electrode and bridge a few times into and out of the agar after the quinhydrone had been sifted over a small area of the surface of the medium. The pH was also determined by using an antimony electrode. This electrode and the bridge were merely inserted into the agar in a Petri dish; the voltage was read as soon as equilibrium was reached; and then the voltage was converted to pH by the use of the equations given by Barnes and Simon (1). Results obtained by the 4 different methods on 2 series of agars prepared at different times are given in table 2.

TABLE 2.—*Comparison of the quinhydrone and the antimony electrodes with the colorimetric method for determining the pH of solid media*

Agar number	pH by colorimetric method		pH by mixing quinhydrone with agar in a mortar		pH by sifting quinhydrone on surface of agar		pH by antimony electrode	
	Series 1	*Series 2*	*Series 1*	*Series 2*	*Series 1*	*Series 2*	*Series 1*	*Series 2*
1	2.0	2.2	2.00	2.13	2.11	2.15	2.24	2.20
2	2.6	3.2	2.60	3.18	2.64	3.19	2.84	3.11
3	2.8	4.4	2.89	4.39	2.93	4.39	3.09	4.82
4	3.8	6.0	3.83	5.86	3.82	5.93	4.16	5.95
5	5.2	6.4	5.48	6.28	5.48	6.28	5.78	6.58
6	6.0	6.6	6.18	6.54	6.18	6.56	6.34	6.66
7	7.0	7.0	6.83	6.90	6.84	6.92	6.99	7.15
8	7.4	7.2	7.15	7.00	7.16	7.07	7.26	7.12
9	7.8	7.4	7.45	7.10	7.45	7.13	7.56	7.31
10		7.6		7.26		7.28		7.39

DISCUSSION

The results given in table 1 show that most of the common culture media used by plant pathologists are not buffered sufficiently so that they can be diluted without appreciably changing the pH. In most cases the pH of the media used was altered by 0.1 to 0.2 of a pH integer when diluted 5 times. Kolthoff (7) has studied the effect of dilution on the pH of buffer mixtures and found that a dilution of 2 times changes the pH of acetic acid-sodium acetate, potassium biphthalate-sodium hydroxide, mono-potassium phosphate-sodium hydroxide, and mono- and di-potassium citrate buffer mixtures a detectable amount. Diluting these buffers 5 times altered the pH almost as much as a similar dilution changed the reaction of the media listed in table 1. An accurate pH determination on many solid media evidently can not be expected or obtained by making a reading on a sample of the medium diluted to the extent that it remains liquid.

Determinations made with the quinhydrone electrode on solid media compare well with colorimetric readings up to neutrality, table 2. Above this point, however, the quinhydrone readings are slightly too low. Agars reading pH 7.2, 7.4, and 7.8 colorimetrically gave readings of 7.07, 7.16, and 7.45, respectively, with the quinhydrone electrode. If the colorimetric readings are accurate, and they probably are more nearly so than determinations made with the hydrogen electrode on diluted media, the quinhydrone electrode values are 0.13, 0.24 and 0.35 pH units too low. Lisse, Jensen, and Tittsler (8) reported that the quinhydrone electrode gave values 0.16 pH lower than those obtained by the hydrogen electrode in diluted nutrient agar of which the pH, as determined by the hydrogen

electrode, was 7.2 to 7.3. Evidently the quinhydrone electrode does not give accurate readings at as high pH values in agar media as it does in buffered solutions, since Biilmann, Klit, and Swaetichin (4) found that correct values were obtained in phosphate mixtures up to pH 7.73, and above this point the values were slightly too low but they were fair even at pH 8.04.

The values obtained with the antimony electrode do not check as closely with the colorimetric readings as those obtained with the quinhydrone electrode. Above neutrality the antimony electrode also gave values which were too low. More trouble was experienced in making readings with this electrode than with the quinhydrone; mainly because it was slower in coming to equilibrium. In general, it was less satisfactory than the quinhydrone electrode.

The electrometric method of measuring the pH of solid culture media that are acid or only slightly alkaline by use of the quinhydrone electrode has several advantages over the colorimetric method. An accurate reading can be quickly made without melting the agar and without any of the difficulties experienced with the colorimetric method due to the turbidity of the media. The electrometric apparatus requires much less attention than the buffer mixtures and standards for colorimetric determinations. Readings can be made on small amounts of medium. For instance, the hydrogen-ion concentration of an agar medium under an organism can be measured by inverting the layer of medium in a culture dish and making the determination on that part of the medium below the organism.

A somewhat serious criticism of the quinhydrone electrode is that the potential is likely to drift. Because of this characteristic it is necessary to make the readings at some definite time. Lisse, Jensen, and Tittsler (8), in an effort to eliminate the effect of the drift, extrapolated the values to zero time. The data given by these authors show that in nutrient agar the pH readings obtained after 1 minute are, on the average, about 0.03 of a unit lower than when they are extrapolated to zero time. Baver (2) found that the first reading made not later than 1 minute after immersion of the electrode was the best one. For most work the first reading, taken not later that 1 minute after immersion, should be sufficiently accurate.

If the reaction of the medium is above pH 7.1, the readings obtained with the quinhydrone electrode will be too low and corrections should be made.

SUMMARY

The effect of dilution on the pH of the following agar media has been studied: Nutrient, corn-meal, Lima bean, bean-pod, prune, and potato-dextrose. Diluting these media with distilled water 5 times, at which dilution all except prune agar were liquid or semi-liquid, altered the pH by 0.1 to

0.2 of a unit as determined by the quinhydrone electrode. An accurate pH determination can not be made on these agar media by diluting them to the point where they remain liquid.

The quinhydrone electrode gave pH readings that compared well with colorimetric readings on solid potato-dextrose agar media from pH 2.0 to 7.1. Above pH 7.1, readings obtained with this electrode were too low. Determinations made with this electrode checked more closely with colorimetric readings than did those obtained with an antimony electrode. The antimony electrode also gave readings that were too low when used in alkaline media.

The quinhydrone electrode furnishes a simple, quick, and accurate electrometric method of determining the pH of acid or slightly alkaline solid culture media. It is much more satisfactory than the colorimetric method over the pH range where it can be used.

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THE USE OF OIL-SOLUBLE COPPER AS A FUNGICIDE

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Copper, as used in the spraying of plants, has been applied almost entirely in standard methods such as Bordeaux or other forms of water-carried copper, the copper being either in a suspension form, as in Bordeaux, or the ammonical-copper solution in water. In the former type the copper is present in a gelatinous form, in the latter as a solution, but restricted in its penetration by reason of the water carrier. Such applications must of necessity deposit the copper on the surface of the leaf and twig with but little possibility of absorption within the tissue of the plant itself. Copper absorption into the leaf from Bordeaux sprays has been noted,[2] but only in minute quantities and particularly through injured cuticle. The literature as a whole indicates surface deposits only[3] rather than penetration into the cellular structure of the plant where the mycelium of most fungi is found. It is generally recognized that, once the germ tube of a spore penetrates into tissue, it then becomes difficult to kill. This difficulty is due primarily to the use of surface deposits of the fungicides commonly selected. To overcome this difficulty it is necessary that we should have fungicides capable of penetrating plant tissue or solvents for fungicides that will penetrate into the tissue of the plant. The principle is similar to the search for chemicals that will kill the bacteria of pear blight within the twig.

The use of oil-soluble copper compounds has been found to accomplish much of this desired purpose and to overcome the criticism of surface deposit of metallic fungicides. It has been shown that most oils, even oil emulsions, penetrate readily[4] through stomata and even through epidermal cells and in this way offer possibilities of carrying oil-soluble toxics into the tissue. To accomplish this purpose, of course, it is necessary to have solutions of copper rather than copper suspensions, as the latter would usually if not in all cases be sieved out by the cell walls. The following experiments indicate that the copper is carried into the plant wherever the oil penetrates and that in this way it is possible to carry fungicides into

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[3] Winston, J. R., and H. R. Fulton. The Field Testing of Copper-Spray Coatings. U. S. D. A. Bul. 785. 1919.

[4] de Ong, E. R., Knight, Hugh, and Chemberlin, J. C. A Preliminary Study of Petroleum Oil as an Insecticide for Citrus Trees. Univ. of California Hilgardia 2, No. 9, 1927.

plant tissue. The organic form of copper known as copper resinate, $Cu(C_{20}H_{29}O_2)2$, is dissolved in a pine-tar oil and the combination made water soluble and diluted as desired. The oil used has been developed especially from the standpoint of plant tolerance and is known to the trade as "Palustrex." It is especially desirable to use oils of a high plant tolerance, for such work as oils that oxidize readily or that naturally carry large amounts of organic acids are too dangerous for use on plants. Copper resinate dissolves readily in this form of pine tar oil and may be blended with petroleum or used in water-soluble form either alone or with petroleum and may also, if desired, carry nicotine where a combination insecticide and fungicide is necessary. Such combinations of copper resinate and Palustrex, as mentioned above, have been in common use in California for the last 3 years, in field work, with increasing popularity and with decidedly less metallic copper than is required to secure similar control with Bordeaux. The inference was that the more perfect distribution both on the surface and within the tissue of the plant of the copper applied gave better control than that applied as a surface application and subject to loss by weathering. To confirm this theory, the following experiments were made:

A heavy Bordeaux spray was prepared to check against the copper-oil spray, each concentrate containing 1.33 per cent of metallic copper. These are, of course, excessive amounts over that commonly used for spray purposes but, since the colorimetric method of determining copper does not detect very minute quantities, it was found necessary to use the undiluted copper-oil spray and to make a correspondingly heavy Bordeaux in order to have for determinations sufficient copper present after given intervals of time. The trial was made both on apricot leaves and twigs and plum twigs in the late fall but before the leaves had fallen. After 30 days, determinations of copper were made both on the surface and on the interior of the twig with the following results: The Bordeaux showed 80 per cent recovery from the surface of the twigs but no copper could be found in the tissue by digesting the twigs after the removal of the surface copper. The oil-coated twigs were still quite oily at the end of the 30-day period because of the heavy application made, and approximately 60 per cent of the copper was recovered from the surface of the twigs; but then, after thorough cleansing in a weak nitric acid solution, a determination of the copper present in the tissue itself showed 21 per cent of the total amount applied, leaving 19 per cent unaccounted for by weathering. It should be noted, however, that the colorimetric method used gives only approximate readings. These findings confirm the theory that oil may be carried into the tissue of the leaf and twig and to such a degree as to be recovered in demonstrable amounts.

The method used in determining the copper present follows that outlined in Bulletin No. 785, U. S. D. A., from which the following abstracts are made:

The reagents used: copper sulfate crystals, 3.928 grams dissolved in 1,000 cc. of distilled water; potassium ferrocyanid, 2 grams dissolved in 100 cc. of distilled water.

Stock dilutions of the copper sulfate solution are prepared each containing one-half cc., 1 cc., 1½ cc., 2 cc., and so on up to 10 cc., in 100 cc. of distilled water. These dilutions would then contain from one-half to 10 milligrams of copper per 100 cc.

Leaves or twigs for testing are collected usually in amounts of 200 gram quantities, each washed separately in 1,000 cc. of distilled water containing 2 cc. of c.p. nitric acid. Colormetric standards are made up of 5 cc. portions of the graded stock solution of copper sulfate containing a few drops of potassium ferrocyanid solution to give typical color reactions. Similar portions of washings of leaf and twig samples may then be prepared with the indicator added. Match the samples of standard copper solutions against those of the washings showing similar color; multiplying this reading by 5 gives a value in milligrams of copper per 100 grams of fresh leaf sample.

To determine copper within the tissue, a digestion method[5] was applied similar to that used in arsenical determinations where apple parings are digested in nitric acid following the addition of sulfuric acid and evaporated down to almost dryness. The residue is dissolved again in a weak nitric acid solution and determinations made according to the previous procedure.

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A BACTERIOPHAGE IN RELATION TO STEWART'S DISEASE OF CORN[1]

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A form of transmissible lysis or bacteriophage has been found in some instances associated with *Aplanobacter stewarti* (E. F. Smith) McCulloch, the organism that causes Stewart's disease of corn. The most favorable material for the isolation of a bacteriophage has been the roots and lower nodes of corn plants killed by the disease, grain from a badly infected crop, and the lower nodes and pith of corn plants that for a time showed the leaf symptoms of Stewart's disease and that later recovered.

The first test for the presence of a phage was made in November, 1933. The basal portion of corn plants that had wilted and dried in midsummer of that year were used, the material minced, being placed in a flask, covered with nutrient broth, and then set aside at room temperature for 48 hours. The liquid was next passed several times through filter paper, and finally, through a Chamberland-Pasteur L3 cylinder, using suction equivalent to about 10 cm. of mercury. To tubes containing 10 cc. of Bacto-nutrient broth, pH 6.8, seeded with 5 drops of a 24-hour-old culture of *Aplanobacter stewarti,* the filtrate was added in graded quantities of 7, 14, and 20 drops. In each set of 5 tubes 1 was reserved for a culture check and 1 for testing sterility of filtrate.

After incubation for 24 hours at 25° C., inhibition was evident in tubes to which the filtrate had been added. Serial passages gradually increased the titer. As the activity of the phage was enhanced, manifested by more complete inhibition or lysis, the amount of filtrate to new cultures was gradually reduced to 3, 5, and 7 drops, regardless of the quantity of medium used. At the 38th passage inhibition was demonstrated at a dilution of 10^{-7}. Even at the point of greatest activity of the phage filtrate, the bacterial cultures were not sterilized by the phage; yet the character of the growth was profoundly changed. No pellicle developed, the broth remained clear for 3 or 5 days, and later a heavy, coarse sediment formed, accompanied by slight clouding of the liquid. All isolations of *Aplanobacter stewarti* were not equally affected by the phage.

Physiological changes in the corn-wilt organism induced by the phage were further demonstrated by the intimate association of bacterial culture and phage filtrate upon potato-dextrose agar. These changes were evidenced by variation or loss of yellow color, increase or decrease of viscosity of bacterial growth, and by reduction or loss of virulence. The titer or state of activity of the phage was found to be an important factor. As the titer increased, inhibition upon solid media became more definite, until

[1] Published with the approval of the Director of the Ohio Agricultural Experiment Station.

finally very little growth occurred. These modifications of bacterial growth, thus artificially obtained, seemed to duplicate the variants or strains of *Aplanobacter stewarti* that were frequently isolated from diseased corn plants. After a mixed culture of bacteria and phage had been grown upon solid medium for 6 generations, the phage was readily reclaimed.

A quantity of seed corn, collected from a badly infected crop in September, 1933, was shelled and stored in the laboratory at room temperature. No difficulty was experienced at any time in isolating from this grain a phage that manifested inhibition for cultures of the wilt organism obtained from diseased plants of the same crop as the seed. This seed, when planted, was found to develop only 5 per cent of infected plants.

It was then decided to test the effect upon naturally infected grain of treatment with a bacteriophage of relatively high titer. Cultures were prepared in quantity, using 500 cc. units of nutrient broth inoculated with 5 drops of a 24-hour-old growth of *Aplanobacter stewarti*. As soon as faint clouding was noticeable, 5 drops of the phage filtrate were added to each culture. After 48 hours' incubation, the lysed cultures were filtered through a Berkefeld cylinder and the liquid used for seed treatment. The grain was soaked in the filtrate first for 24 hours in an ice-box at 50° F. and then for the same period at room temperature. After draining and partially drying in air, the corn was planted.

Eighteen lots of commercial seed, obtained from different seed firms, were used in these tests. Plots were located upon a great variety of soils. The average amount of infection where phage-treated seed was used was 1.4 per cent, whereas the average infection for the nontreated check plots was 18 per cent. Insects, including chinch bugs, 12-spot cucumber beetles, and flea-beetles, were present in all plots. There appeared to be less spread of the disease in the plantings from seed treated with the phage than in the check plots. In the treated plots the amount of infection ranged from none to 4 per cent, whereas untreated plots varied from none to over 40 per cent.

Examinations have been made of corn plants that exhibited mild infections or that appeared to be in a stage of recovery. In many cases the organisms isolated were found to have a phage associated with them. When mixed cultures of bacteria and phage were injected into healthy plants, in some instances typical leaf striping occurred, followed by recovery; in others, no symptoms developed.

The facts presented suggest that a bacteriophage may play an important rôle in reducing losses due to Stewart's disease. The presence of the phage in plants recovering from the disease and in seed that, although taken from diseased stock, produced relatively few diseased plants lends support to the idea. The most important evidence, however, is gained from the results of seed treatment, by which the disease was reduced in every case to a minimum.

Rate of Increase in Area of Apple-Spur Leaves.[1]—The period between the delayed dormant and the calyx- or petal-fall stage is generally accepted as the critical period for apple scab (*Venturia inaequalis* (Cke.) Aderh.) infection and control. It is during this period that the areas of the apple-spur leaves are undergoing their most rapid development. Since the unfolding and increase in area of apple leaves has an effect on the subsequent distribution of spray material on the leaf surface, which may affect the degree of protection afforded, we determined to measure the area of apple-spur leaves at 2 and 3 day intervals during this critical period.

One hundred approximately uniform flower spurs, each generally bearing 11 leaves, which is characteristic of the variety, were selected for each determination from 3 Rome Beauty apple trees from April 25 to May 10. Each sample of 100 spurs was divided into several equal lots and the area of the leaves of each lot determined after removing them from the spurs and placing them in the measuring apparatus. In this manner it was possible to observe the variations in leaf area that were present. The areas were

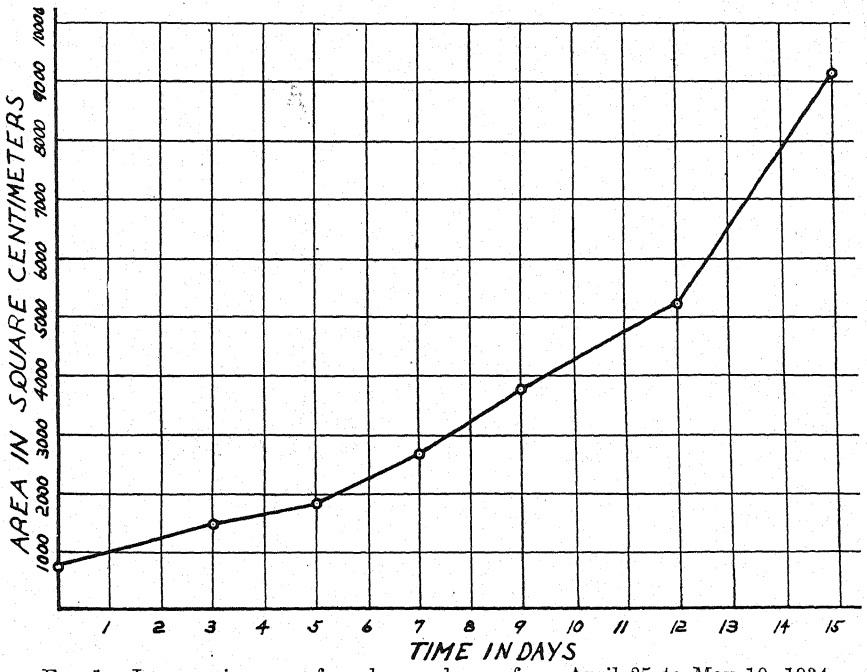


FIG. 1. Increase in area of apple-spur leaves from April 25 to May 10, 1934.

[1] The writers wish to express their sincere appreciation to C. L. Burkholder of the Department of Horticulture, Purdue University Agricultural Experiment Station, for cooperation in this work.

measured by the photoelectric device described by Withrow,[2] which is accurate to approximately 1 per cent. The greatest variation in area between individual lots of the same sample was approximately 10 per cent. Since a variation in the leaf area between spurs occurs, care in sampling and the use of a large number of spurs is necessary in a determination of this kind.

In figure 1 the area in square centimeters of 100 spurs is plotted against time in days. The rate of increase in total area of the leaves from April 25 to May 10 became progressively greater. During the period of 15 days the total leaf area increased to 9 times the original value. The growth of flowering apple spurs does not progress at the same rate in all seasons. For example, in 1933, the rate of growth was slower than in 1934, and 21 days were required for the passage of fruit spurs through similar stages of development. Fruit spurs and leaves representative of the samples taken April 25, May 2, and May 10, 1934, are shown in figure 2.

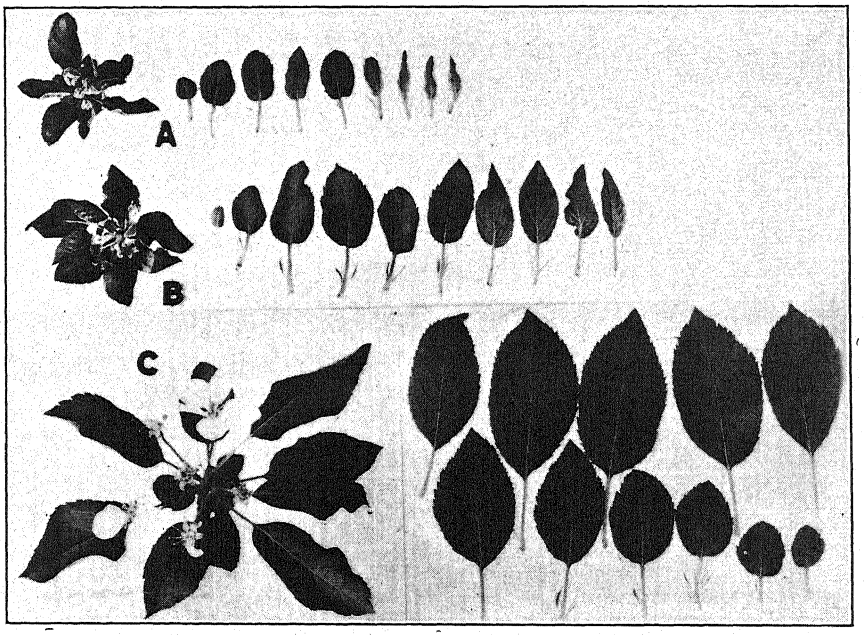


FIG. 2. Rome apple spurs and leaves representative of the samples taken on April 24, May 2, and May 10, 1934. A. Spur in the prepink stage, having only the first 5 leaves on the spur unfolded. B. Pink stage, 7 days later, when the last leaf has unfolded and may be covered with spray. C. Rome spur in the early petal-fall stage taken 15 days later than A. ×0.2.

[2] Withrow, R. B. A photoelectric device for the rapid measurement of leaf area. Unpublished. Manuscript on file in the Department of Horticulture, Purdue University, La Fayette, Indiana.

The rapid rate of unfolding and increase in area of the young leaves, particularly prior to the pink-bud stage, emphasizes one of the difficulties encountered in practice in protecting the foliage from infection with sprays applied during this early period.—R. C. BAINES and H. M. BENEDICT, Purdue University Agricultural Experiment Station, La Fayette, Indiana.

Hybridization Between Sphacelotheca sorghi and Sorosporium reilianum.—Recently the writers succeeded in crossing the covered kernel smut of sorghum, *Sphacelotheca sorghi* (Link) Clinton, and the head smut of

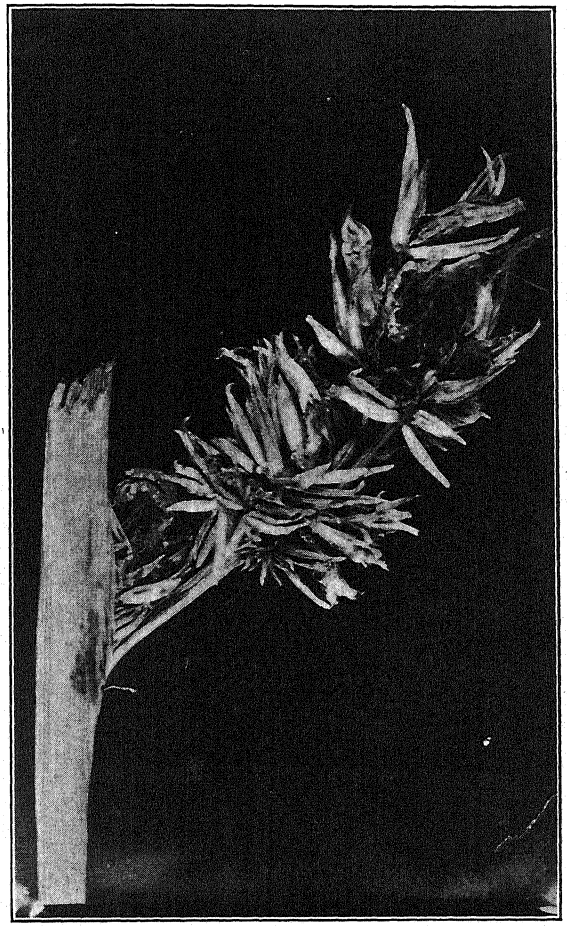


FIG. 1. A head of Waconia orange sorgo infected with hybrid smut produced by injecting hypodermically into the young plant a monosporidial culture of *Sphacelotheca sorghi* combined with a monosporidial culture of *Sorosporium reilianum*. Note the horn-like sori and floral proliferation.

sorghum, *Sorosporium reilianum* (Kühn) McAlpine. Two monosporidial lines of *Sphacelotheca sorghi* and four of *Sorosporium reilianum* were grown singly in flasks of potato-dextrose broth, after which sorghum plants were inoculated by means of a hypodermic syringe with the broth cultures of single lines and with paired combinations. None of the single lines caused infection, but, after an incubation period of from 7 to 10 days, chlorotic flecking such as commonly appears on sorghum plants injected with combinations of sexually compatible monosporidial lines of *Sphacelotheca sorghi* or of *Sorosporium reilianum* appeared on 2 lots of plants which had been injected with different combinations between monosporidial lines of the 2 species. When the plants approached maturity, sori containing chlamydospores were developed in the inflorescences of some of the plants that had previously shown chlorotic flecking.

Sori and spores both appeared to be somewhat intermediate in type between those of *Sphacelotheca sorghi* and *Sorosporium reilianum*. The sorus tissue developed chiefly from the ovaries and closely associated floral parts, as is the case with *Sphacelotheca sorghi,* but sori were much longer and more irregular in shape than those of this smut. The leathery white peridium covering the sori and the abundant floral proliferation resemble those of *Sorosporium reilianum* (Fig. 1). Chlamydospores were somewhat larger than those of *Sphacelotheca sorghi* but within the range for those of *Sorosporium reilianum*. The walls were finely echinulate, much less distinctly so than those of *Sorosporium reilianum,* but not smooth like those of *Sphacelotheca sorghi*. In this respect the new smut, therefore, is also intermediate in type. The chlamydospores germinate abundantly, producing very long, slender promycelia somewhat like those of *Sphacelotheca sorghi*. Sporidia are produced sparsely and resemble those of *Sphacelotheca sorghi,* being more slender and tapering than is typical for those of *Sorosporium reilianum*. Frequently, some promycelia produce numerous hyphal branches with very few or no sporidia.

Studies on cultural characters, pathogenicity, and genetics of the new hybrid are now under way and will be reported later.—LEON J. TYLER and CLYDE P. SHUMWAY, University Farm, St. Paul, Minn.

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THE HOST RANGE OF THE SOUTHERN CELERY-MOSAIC VIRUS

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Farmers in certain winter truck-growing regions of Florida have long been troubled with a serious mosaic disease of celery. The writer (8) described the virus causing this mosaic, found it was new, and named it *Celery virus 1.* The exact geographical distribution of the virus is not yet known, though Doolittle and Wellman (2) have reported celery with the same mosaic symptoms from Florida, Ohio, New York, Wisconsin, and California. The writer (9) also has reported it from Cuba. Two investigators, Kunkel (5) and Cook (1), have reported virus-diseased Commelina plants from other tropical regions.[1]

Doolittle (*cf.* Doolittle and Wellman (2)) was the first to find that the monocotyledonous weed, *Commelina nudiflora* L., was a common host of the virus. The writer has since then reported (8) a fairly large host range, certain portions of which further proved the distinctive properties of *Celery virus 1.* Encouraged by such knowledge, additional inoculations were attempted on both dicotyledonous and monocotyledonous plants. Proof of infection and study of the diseases produced has been secured on several species of Gramineae (9) and 2 species of Musa (10). In connection with the studies on field dissemination of the virus (11) careful observations were made on new weed and cultivated hosts with apparent virus infections in nature and these were further checked experimentally. In work upon methods to control the virus much attention has been given to eradication measures, some preliminary results of which have been reported (7). As the control problem has advanced it has become important to know more fully the commonly affected weeds and ornamentals. In the course of these studies it has become evident that the virus attacks a much larger group of economically important hosts than was at first considered likely. For all these reasons special attention was given to this problem, and this paper reports the results of the investigations.

[1] Carter has stated very recently (Carter, W. Mechanical transmission of two viruses to pineapple. Phytopath. 25: 10. 1935.) that "Mosaic of *Commelina nudiflora* in Hawaii is apparently identical with that described from Florida."

MATERIALS AND METHODS

The source of virus (*Celery virus 1*) material used in these studies was exactly the same as that originally described by the writer (8). All aphids, *Aphis gossypii* Glover, used for inoculations in these studies were from colonies maintained by S. P. Doolittle of the Bureau of Plant Industry. The specific names of experimental plants were determined with as great care as possible and are included in the complete host range reported (Table 5 (p. 394)) and the list of apparently nonsusceptible hosts (p. 399). The vegetable and ornamental plants were all from seeds of named varieties from reputable seedsmen. The weeds were largely from around vegetable fields in Florida, and the specificity of susceptible species, with the exception of the Phytolaccas and *Cucumis anguria,* were determined by S. F. Blake of the Bureau of Plant Industry. In the miscellaneous plant list, seeds of small grains and teosinté were obtained through A. G. Johnson of the Bureau of Plant Industry. With the exception of a few packets purchased from seedsmen, all corn, or maize, seed was secured through J. G. Dickson of the Bureau of Plant Industry and University of Wisconsin. Bulbs of flowering ornamentals were obtained through Freeman Weiss of the Bureau of Plant Industry. The solanaceous plants used were grown from seeds supplied by F. O. Holmes of the Rockefeller Institute for Medical Research, except the Daturas and Broadleaf tobacco which were from S. P. Doolittle.

Whereas the majority of the inoculations of plants were made in the greenhouse at Sanford, Florida, a few of the reported tests were carried on in greenhouses at Arlington Experiment Farm, Rosslyn, Virginia, in New York and also in Wisconsin (8) while the writer was determining the identity of *Celery virus 1.* With the exception of a few hosts, inoculation studies were repeated more than once and under varied conditions.

Original plants of *Commelina nudiflora* were kept for source of virus. Aphids were colonized on these and transferred to young White Spine cucumber, *Cucumis sativus,* and Golden Self Blanching celery, *Apium graveolens,* plants. On the day the vein-clearing symptoms became severe, leaves with these symptoms were removed, chopped up, ground to a pulp in previously boiled porcelain mortars, and the juice squeezed out through new cheesecloth into boiled glass jars. Bits of new cheesecloth were wetted with these viruliferous juices and were rubbed over the washed leaves of plants to be tested for susceptibility to the virus. Every series of plants inoculated was rubbed with a separate piece of juice-moistened cloth, and once a piece was used for inoculation it was discarded. Hands were washed with soap and water and dried between inoculating each set of plants. In 1 to 5 minutes after inoculation, excess of juice that had been rubbed

onto the plants was washed off, and in most cases the plants were protected from exposure to severe sunlight for about 18 hours.

Viruliferous aphids were secured in the following manner: Diseased cucumber and celery plants were placed under cheesecloth cages. Leaflets with virus-free aphids from colonies maintained on healthy cucumber and celery plants were transferred to the diseased plants and the aphids were allowed to feed on virus-affected leaves from 5 to 14 days. It was early found that, besides the tedium involved, aphids transferred with camel hair brushes were not consistent in their ability to transmit the virus. If allowed to crawl away from diseased leaf pieces onto healthy foliage, however, infection occurred more generally. In consequence, bits of diseased leaves, having 15 to 40 undisturbed aphids on them, were clipped from colonized diseased plants and fastened in the foliage of the plant to be tested for susceptibility. In the case of banana plants from 75 to 150 aphids were used (10) to transmit the virus.

Plants infested with viruliferous aphids were kept in cheesecloth cages for 5 to 10 days, then sprayed with aphicides a number of times, and finally removed from the cages when all aphids were killed. Plants inoculated by the leaf-rubbing method also were sprayed at intervals and all inoculated plants were watched 2 months for disease production before being discarded. Precautions were taken to insure certainty that leaf injuries, sustained from leaf-rubbing methods, or nonviruliferous aphid-feeding marks, were not mistaken for primary lesions. From 5 to 55 plants of every inoculation series were always kept untouched, except that they were sprayed, for control purposes. Occasionally, aphids escaped from inoculation cages and in a few cases a virus infection appeared in the control plants as well as in the inoculated series. On such occasions series connected with these occurrences were discarded, all diseased and healthy plants, whether they had been inoculated or not, were destroyed and the inoculations were repeated on plant material obtained from an absolutely new source of seedlings.

With a few exceptions all hosts that became diseased after inoculation were tested by juice or aphid transmission to celery and cucumber seedlings for the presence of the virus. In most of these cases the originally inoculated leaves were labelled and their juices were tested separately from the other leaves, which either remained symptomless or showed evidence of systemic invasion. While not every plant that gave negative results to celery-virus inoculation was tested, a few plants of a large majority of the apparently virus-free species were tested.

Parallel with the greenhouse host-range experiments, field observations were conducted to corroborate these studies, and to gain knowledge of dissemination of this virus (11) and its natural occurrence. Diseased foliage

from any ornamental, weed, or vegetable plant found with symptoms of virus attack in the field was brought in and tested for presence of *Celery virus 1*. This phase of the investigations included diseased material on many hosts from widely separated regions in Florida. After positive results from tests of naturally diseased plants had been obtained the first time, a second lot of material was secured and tests repeated.

EXPERIMENTAL RESULTS

Vegetable Crops Diseased by the Celery Virus

The celery virus was described by the writer (8) from severely affected celery. Before the virus had been described, Doolittle and Wellman (2) had determined that celery plants could serve as source of inoculum to produce virus infection of tomato, *Physalis pubescens* (husk tomato) and cucumber. In addition to the above vegetables the writer found (8) that the red garden beet, Tabasco pepper, sweet peppers, Cocozelle squash, summer crookneck squash, muskmelon, and watermelon were susceptible vegetable-crop hosts, and later reported (9) that sweet corn and sweet potatoes were likewise susceptible to the virus. In a recent paper (11) the writer has described natural occurrence and dissemination of this virus in fields of Golden Self Blanching celery, Golden Summer Crookneck squash, Cocozelle squash, California Wonder pepper, World Beater pepper, and sweet corn.

In the accompanying table (Table 1) is a list of all vegetable crops that have proved susceptible to *Celery virus 1* under conditions of these investigations. The common English names have been used with numbers indicating the Latin binomials designating their species (Table 5). A great many vegetables tested for susceptibility proved apparently immune from this virus, as may be noted by reference to the list (p. 399) appended at the close of the presentation of experimental results in this paper.

One of the surprising features of the host-range studies has been the fact that so many vegetable crops are susceptible to the celery virus. In some cases the symptoms of this virus attack differ so greatly in separate species that it is believed of interest, as well as of importance, to discuss a few such reactions in detail on given crops.

Celery: The southern celery mosaic (*cf.* (8) (2)) disease is most notable in the fields when plants are approaching the proper size for harvest. The rows appear uneven and present a ragged appearance to the field, especially around the edges, though it may extend throughout the field. Diseased plants are stunted. They have a badly mottled and yellowish aspect, leaflets are twisted and flattened towards the ground, and the petioles and leaf rachises are often darkened throughout; though sometimes scattered buff, almost salmon color, water-soaked spots and sunken streaks appear on them. The markedly stunted appearance of plants is due somewhat to

TABLE 1.—*Results of studies on vegetable crops found susceptible to the southern celery-mosaic virus, including symptoms and field occurrence (Compare with Table 5 for species names, number of plants used, and recovery of virus)*

No.[a]	Vegetable Crop	Inoculation methods	Symptoms[b]	Occurrence in field
21	Beet (Red Garden)	Aphid, rubbing	LR–M–*M*–S	+
35	Carrot	" "	.....N–M–Y	+
42	Celeriac	Rubbing	LO–S–M–N–Y–D	
39	Celery (Golden types)	Aphid, rubbing	LO–S–M–N–Y–D	+
40	Celery (Flavoring)	Rubbing	LO–S–M–Y	
41	Celery (Green Pascal)	Aphid, rubbing	LO–S–M–N–Y–D	+
22	Chard, Swiss	Rubbing	LN–M–*M*	+
2	Corn, Sweet	Aphid[c]	LY–*S*–S–M–N	+
83	Cucumber	Aphid, rubbing	LN–M–S–N	+
36	Dill	"	.....S–Y	
55	Eggplant	" "	LN–M–*M*	+
37	Fennel	"	.....S–Y	
59	Groundcherry, Common	" "	.....M–S–D	+
58	Groundcherry, Strawberry	Rubbing	.....M–S	
84	Muskmelon	"	LN–M–S–N	
26	New Zealand Spinach	Aphid, rubbing	LW–M–*M*	+
16	Onion	Aphid[c]	.....M–S–D	+
38	Parsley	Aphid, rubbing	LY–M–N–Y–D	+
43	Parsnip	Rubbing	LO–M–N–Y	
66	Pepper, Sweet	Aphid, rubbing	LN–M–N–S	+
77	Pumpkin	" "	LW–M–S–N–D	+
23	Spinach	" "	LN–N–Y–M	+
79	Squash (Cocozelle)	" "	LN–M–D–Y–S–N	+
78	Squash (Golden Summer Crookneck)	" "	LN–M–D–Y–S–N	+
80	Squash (Hubbard)	Rubbing	LD–M–D–S	
47	Sweet Potato	Aphid[c]	.....M–D–S	+
56	Tomato	Aphid, rubbing	LD–Y–M	+
30	Turnip[d]	Rubbing[e]	LN	
81	Watermelon[f]	Aphid, rubbing	LN–M–Y–S–N	+[f]

[a] These numbers refer to host species identically numbered in table 5.

[b] L refers to primary lesions: D-diffuse, N-necrotic, O-none developed, R-reddish, W-whitish, Y-yellowish, ...-uncertain of production. Other single letters refer to systemic symptoms: D-distortion, M-mottling, *M-masking,* N-necrosis, S-stunting, *S-stripe,* Y-yellowing.

[c] Leaf-rubbing methods failed to produce infection.

[d] Credit should be given at this point to T. J. Grant, who, while in a collaborator's capacity (8), first observed independently primary lesions caused by the celery virus on turnip leaves. The writer secured the results cited here on tests involving one variety in Florida.

[e] No tests with aphid inoculation methods produced infection.

[f] Since preparing these data for publication naturally infected volunteer watermelon plants have been observed in a field near Sanford, Florida.

shortened leaf petioles and rachises, but in greatest measure to a downward curvature of those foliage parts. The dark or streaked petioles and rachises are at first brittle and easily broken; later, becoming more withered and necrotic and finally decaying.

Inoculation studies have shown that the first symptom of southern celery mosaic on young leaves is a faint vein-clearing pattern, which occurs under the most favorable greenhouse conditions in about 6 days, although it may take 10 to 40 days for it to appear in the field. The vein-clearing pattern on leaves usually changes in about 10 days to a brilliant yellow mottling that, about a month later, is not so marked. Secondary systemic symptoms in older leaves result in yellowish oak-leaf patterns which in some cases take the form of continuous zig-zag bands across the leaf lamina, and in other cases are markedly irregular and may break up into chlorotic blotches and spots.

Sweet Pepper: Plants, severely diseased with *Celery virus 1,* have been studied (11) in the field. They appear badly stunted, leaves are small and distorted but without filiformity, with a grayish dull color making the plants seem starved, blossoms drop, if fruit has set before the plant is infected it becomes malformed, and a mild mottling coupled with a finely ridged moiré pattern occurs on some of the leaves. The disease often starts at the edges of the fields a few days after transplanting, and, once established, usually spreads rapidly.

Under favorable experimental conditions, inoculations on California Wonder peppers, either by aphids or leaf-rubbing methods, often resulted in dark primary lesions that spread downward to the stem and caused systemic symptoms. Primary lesions have been observed within about 4 days after inoculation, and systemic infection resulting in crookneck and vein-clearing symptoms occurred a day or two later.

Cucumber and Summer Squashes: Celery virus 1 attacks cucumber and summer squash plants with about equal severity. When observed in the fields (11), the disease is a vigorous mosaic causing striking stunt and necrosis, and mottling with crumpling of the leaves. The virus usually is found first in plants along the edges of fields, spreading rapidly inwards. Plants may be affected at any time while they are growing, from early seedling stage to when they are mature, bearing fruits ready to harvest. If infection occurs before blossoms are mature, fruits do not set; but when disease occurs a little after this stage, badly stunted, discolored, and slightly malformed, unsalable fruits may be produced.

When cucumber and squash seedlings were inoculated by the leaf-rubbing method, round, sunken, primary lesions were produced on cotyledons, and irregular diffuse primary lesions were produced on leaves in about 3 to 10 days after inoculation. The lesions on Cocozelle squash cotyledons were larger than those on White Spine cucumbers. On both Cocozelle and Golden Summer Crookneck squashes they produced general necrosis of the cotyledons and caused systemic symptoms 2 to 3 days after the lesions were noticed. In cucumbers the reaction was nearly the same, though general

necrosis of cotyledons did not take place until several days had passed. Within 2 weeks after the first leaf mottling occurred, there was little difference in severity of infection in either squashes or cucumbers.

Eggplant and Tomato: The Florida Highbush eggplant was found on a number of occasions infected with the celery virus in the field. The same is true of Globe and Marglobe tomatoes. Diseased plants were slightly stunted, the leaves were mottled in a mild and inconspicuous manner, and, though fruits were somewhat reduced in number and size, they were harvested and the disease caused little important loss in production.

Black Beauty eggplants were inoculated in the greenhouse by the leaf-rubbing method. The first symptoms to appear were vein-clearing, and chlorosis, 10 to 14 days after inoculation. About 6 days later distinct, black, irregularly shaped primary lesions were visible on originally inoculated leaves. Aphid inoculations gave the same results. These experiments were repeated a number of times, and black primary lesions nearly always appeared several days after systemic symptoms were noted on the youngest leaves. No distinct primary lesions were ever observed on Florida Highbush eggplant. On Globe and Marglobe tomatoes, irregular, diffuse, light colored primary lesions occurred occasionally in inoculated leaves, and these symptoms became evident some days after vein-clearing and characteristic mild systemic mottling were established symptoms. Occasional masking of systemic symptoms occurred in eggplant.

Sweet Corn: Corn plants that are conspicuously affected with celery virus infection in the field (9) (11) are stunted, and have leaves that are striped with long but narrow bright yellow bands, running along the veins. The leaves tend to split, may have necrotic streaks, and are of a generally chlorotic appearance. Young seedlings may be killed outright.

Repeated attempts have failed to infect Country Gentleman sweet corn plants by leaf-rubbing methods. Viruliferous aphids, however, have proved a potent means of inoculating seedlings. Primary lesions result from aphid inoculations. It is believed by the writer that his description (9) of these lesions is the first report of such virus-infection phenomena on monocotyledonous hosts. Primary lesions occurred under greenhouse conditions in about 3 days after aphids had fed on the young leaves, and were found with greatest frequency on the first seedling leaf, though they appeared on the second, third, and fourth leaves as well. The start of systemic symptoms consisted in the presence of elliptical spots on the leaves shortly after primary lesions were evident, and a mottling also was often found. These markings gradually changed and coalesced, concentrating the chlorotic effects along veins until within a month's time striping occurred. The stem tissues were affected, the internodes did not lengthen normally, and the plants were severely stunted.

Beet: Garden beets which were naturally diseased with celery virus have not been easy to find in the field. The diseased plants have in some cases a stunted growth, in which the leaves are glazed and held stiffly upright, and a red but mild mottling pattern occurs. At times plants which seemed symptomless but were next to diseased plants were found to contain the virus when their foliage parts were used as inoculum on test plants.

Crosby Egyptian beet plants were inoculated by either leaf-rubbing or aphid methods, and in all cases non-necrotic primary lesions appeared in about 3 days. These lesions were in the form of reddish rings on a green background. Systemic symptoms, which appeared in some cases, developed a number of days after the primary lesions. It was noticeable in the experimental plants that the petioles were brittle in character, and the leaf tissues were in general more thickened and stiff.

Watermelon: The writer has not seen plantings of watermelons growing in regions where he knew the celery virus existed. Kleckley Sweet watermelon plants were grown in the greenhouse, however, and infection by aphids or rubbing methods produced black primary lesions on both cotyledons and young leaves in around 7 days. Symptoms of systemic infection occurred usually a day before primary lesions developed and consisted at first of a light vein-clearing in young leaves. This type of marking faded out, however, the leaves remained small and darker green, mildly but not markedly mottled, and the stems were brownish and woody with short internodes. Plants were considerably stunted and brittle, and one of the striking effects of the disease in the greenhouse was inhibition of flower and tendril formation.

Sweet Potato: The writer has observed sweet potato plants diseased with celery virus in the field in Florida and also has found it (9) in Cuba. Under common cultural practices its presence on sweet potatoes is not especially conspicuous. In the field a mild mottled pattern usually develops, which towards harvest time can hardly be recognized as of virus causation.

Sweet potato plants of the Porto Rico variety were inoculated by aphids and leaf-rubbing methods. The latter technique failed to produce infection, but aphids were successful in transmitting the virus. No primary lesions were produced, but vein clearing appeared in about 7 days after placing aphids on the plants. The vein clearing finally disappeared and the next, younger, leaves to show infection were severely marked with a not very brilliant but distinct mottling characteristic of virus infection on this host. The plants were stunted and produced small roots.

Onion: Onion seedbeds near fields of celery badly diseased with mosaic were examined, and onion seedlings were found which were stunted, and the leaves were generally chlorotic with yellowish markings of rounded and irregular ring shapes. These plants, upon being tested for virus content, proved to be diseased with the celery virus.

Crystal White onion sets and seeds were planted in the greenhouse and the young plants and seedlings used for inoculation experiments. No plants that had been grown from sets were infected, whether inoculated by aphids, needle, hypodermic injections, or the leaf-rubbing method. Upon inoculating very young seedlings, leaf rubbing did not cause infection, but aphid inoculations were successful in causing disease. No primary lesions were observed. The first visible indication of infection appeared as a number of light chlorotic streaks on young leaves. The yellow spots and rings found on older infected plants in the field appeared in diseased plants in the greenhouse. In 6 weeks' time after inoculation, diseased plants had become yellowed, stiff, brittle, and somewhat stunted.

New Zealand Spinach: In several instances celery-virus-diseased New Zealand Spinach plants were found in the field. If diseased weeds had not been adjacent to patches of the vegetable, the New Zealand Spinach plants would not have been believed to be diseased. Symptoms of disease in the field are largely mild mottling, and a slight stunting, and in some cases apparently symptomless plants served as a source of celery virus when juices from them were tested on cucumber cotyledons.

Both leaf-rubbing and aphid inoculations caused inconspicuous, whitish, primary lesions. These were visible at about the same time systemic symptoms appeared. On inoculated plants a mild mottle pattern was produced on the leaves, together with slight stunting, though in many cases no symptoms of any sort occurred.

ORNAMENTAL PLANTS INFECTED BY THE CELERY VIRUS

In selecting the ornamental plants thought necessary to be included in these host-range studies, the first group to be experimented on were those species and varieties found naturally diseased in the Sanford district. The work was then extended to others grown in the region but that apparently remained healthy. A few species commonly grown only in the northern part of the United States also were studied.

In table 2 are listed 23 ornamental host plants that proved susceptible to *Celery virus 1* inoculations. Of these susceptible plants, 10 seemed of particular interest.

Petunia: Rosy Morn petunia plants were found in many gardens, severely affected by the southern celery-mosaic virus. Such plants had mottled leaves, which in a few instances had developed necrotic areas. The stem internodes were much shortened, and under certain conditions distinct necrotic streak symptoms appeared on them. There was often some distortion of the leaf lamina, though no shoestring effects occurred, and in many instances no blossoms developed. In the inoculation experiments, both leaf-rubbing and aphid inoculations caused primary lesions. These

started as small, whitish punctations that enlarged rapidly, becoming slightly yellow, and finally water-soaked and necrotic. Systemic infection symptoms on leaves appeared several days after development of primary lesions, and consisted first of typical vein clearing, and later severe mottling and stunting, which finally ended with necrosis in some cases that extended into the stem tissues. Flowers that showed virus effects were of particular interest. In some cases they were only partially developed being unable to open up completely. Other plants bore flowers that opened normally but were mottled with streaks and concentric irregular rings.

Periwinkle: Symptoms of celery-virus attack on periwinkle plants in the field were frequently observed. Periwinkle, grown as an annual in the North, is treated as more or less perennial in Florida and often manages to exist for years with fairly severe virus symptoms on its leaves. These symptoms in the field are largely confined to marked mottling, and slight stunt-

TABLE 2.—*Results of studies on the ornamental plant hosts that proved susceptible to Celery virus 1 (Compare also with Table 5 for species names, number of plants used, and recovery of virus)*

No.[a]	Ornamental Plant	Inoculation methods	Symptoms[b]	Occurrence in field
89	African Marigold	Rubbing	.....S–D–M	+
57	Belladonna	"	.....M	
51	California Bluebell	"	.....M–N	
50	Chinese Forget-me-not	"	LO–M–D–S	
14	Easter Lily	Aphid[c]	LN–M–S–Y	+
88	French Marigold	Rubbing	.....S–D–M	+
90	Floras Paintbrush	Aphid, rubbing	LO–*M*	+
33	Geranium	Aphid[c]	.....M–S	+
28	German Catchfly	Aphid, rubbing	.....M–S–D	
49	Gilia	Rubbing	.....S–M	
15	Golden Lily	Aphid[c]	.....M–N–S	
27	Iceplant	Rubbing	LN–M–S–D	
29	Larkspur	"	LN–Y–S–M	+
44	Madagascar Periwinkle	Aphid, rubbing	LO–M–S–D	+
46	Morning Glory	Aphid	.....M–S	+
34	Nasturtium	Rubbing	LD–O	
74	Petunia	Aphid, rubbing	LN–S–M–N–D	+
52	Phacelia	Rubbing	.....S–N–M	
48	Phlox	" [d]	LC–O	
75	Snapdragon	Aphid, rubbing	LW–Y–S–M	+
76	Sweet Scabiosa	Rubbing	.....M	
12	Wandering Jew	Aphid[c]	LO–M–S	+
87	Zinnia	Rubbing	LO–S–M–D–Y	+

[a] These numbers refer to host species identically numbered in Table 5.

[b] L refers to primary lesions: C-chlorophyll retention on yellowed leaves, D-diffuse, N-necrotic, O-none developed, W-whitish, ...uncertain of production. Other single letters refer to systemic symptoms: D-distortion, M-mottling, *M-masking,* N-necrosis, O-no symptoms and no virus recovered, S-stunting, Y-yellowing.

[c] Rubbing methods of inoculation did not produce infection.

[d] Aphid inoculations did not produce infection.

ing. Aphid and leaf-rubbing inoculations were made on plants grown under experimental conditions, infection occurred but no primary lesions were observed. The first systemic symptom was a peculiar streaked mottling on the leaves, which later increased in severity. Disease resulted in downward curvature of the leaves and shortened internodes; in some cases no flowers were produced. Where flowers did develop they were small, though not noticeably malformed.

Larkspur: Annual larkspur plants, infected in the field with the celery virus, were markedly stunted and chlorotic and did not produce flowers. Both juice and aphid inoculations proved successful in producing the disease experimentally. Diseased plants were much flattened in growth and had a rosette appearance; leaflets chlorotic, curled down, and in a few cases mottled with light green.

Zinnia: On zinnias in the field the celery virus disease causes severe stunting and mottling. Experimental inoculations did not produce primary lesions, though severe systemic mottling, distortion, chlorosis, and stunting occurred. The colors of diseased zinnia flowers are abnormal with tan and dirty grey markings on the distorted, irregular, and stiff petals. The flower stalk is shortened and the numbers of flowers produced much reduced.

Easter Lily: Both the Easter lily and the Golden lily were found to be susceptible to aphid inoculation with the celery virus. Diseased Easter lily plants were found in abundance in fields, and in all cases the relationship of infected plants and diseased wild hosts was distinctly observed. The Easter lily was found to be affected by at least one other virus disease in Florida, but this trouble differed in symptoms, did not spread rapidly, and was observed on young stock as soon as it was above ground. The celery-virus disease apparently did not occur in the bulbs, but appeared only after aphids had fed on the plants in the field. Celery-virus diseased Easter lily plants are stunted, with leaves flattened out and grey, often with small necrotic spots on them. Flower development is sometimes inhibited. When flowers unfold, they may be small and greenish in color but do not split or develop malformations.

Wandering Jew: Plants of the common purple wandering jew were observed naturally infected with the celery virus in 2 gardens. The diseased plants were not easily discovered but were somewhat stunted with slight twisting of leaves, and a light colored mottling interrupting the broad purple stripes on their under surfaces. Healthy plants were inoculated in the greenhouse and the use of viruliferous aphids was the only successful method of infecting the plants. No primary lesions were produced, and the typical systemic symptoms seen on diseased plants growing in outside gardens resulted.

Snapdragon: Snapdragon plants affected with celery virus were found occurring naturally in the field. They appeared somewhat chlorotic, stunted, and mildly mottled. Inoculation of plants in the greenhouse by rubbing leaves with viruliferous juices produced whitish primary lesions. Aphid inoculations seemed to cause no primary lesions but resulted in systemic infection.

Iceplant: Infection of iceplants was obtained through leaf-rubbing methods. In these plants the first symptom consisted in the manifestation of grey primary lesions, which rapidly became diffuse and were somewhat necrotic. The thick, fleshy leaves of this plant were inoculated on their ventral surfaces. The necrotic appearance of primary lesions did not extend to the dorsal side of the leaf. Systemic-mottling symptoms, which developed on other leaves, were noted on both sides of leaves systemically infected. At times some of these leaves were considerably distorted; under other conditions they were simply smaller than normal.

Phlox: Phlox was studied in the field for natural infection, but nothing of the sort was observed, even in beds surrounded with infected weed hosts. Seedlings grown in the greenhouse were inoculated with aphids but the trials failed, while leaf-rubbing methods produced a few scattered primary lesions. These lesions were neither large nor necrotic. They were first whitish in appearance, but later recovered their greenness, retaining their chlorophyll when the rest of the leaf yellowed and dropped.

Floras Paintbrush: The juice from a few wild plants of Floras paintbrush was shown to contain *Celery virus 1* when inoculated into test hosts. This inoculation was repeated a number of times with consistent results. Such plants were perhaps at first very slightly stunted, though they grew and flowered normally later on. When inoculation studies were performed in the greenhouse, the juice from some inoculated plants still contained the virus 3 weeks after leaf-rubbing or aphid infections, and none of the untouched plants had infectious juices. Taken by themselves, the inoculated plants appeared symptomless from the very first, and grew, flowered, and fruited in a seemingly perfectly normal manner. This was a species that, under the conditions of the experiments, was judged by the writer to be a good example of a masked carrier.

WEED HOSTS OF THE CELERY VIRUS

In the Sanford region it has been of considerable importance to know what wild plants growing around cultivated areas are capable of acting as hosts of the celery virus, since one of the methods of control of this virosis of celery (7) has been the removal of these weed hosts from around celery fields. Although one weed, the wild wandering jew, *Commelina nudiflora,* has been considered the principal reservoir-host of the southern celery

mosaic, it has become increasingly evident that other weeds are also of great significance in the spread of the virus to other crops. In table 3 are found the results of studies of weed hosts of *Celery virus 1.*

TABLE 3.—*Results of studies on weed hosts of the southern celery-mosaic virus (Compare also with Table 5 for species names, number of plants used, and recovery of virus)*

No.[a]	Weed host	Inoculation methods	Symptoms[b]	Occurrence in field
45	Bindweed	Aphid	.....M–S	+
32	Crane's-bill	" [e]	.....M–D–S	+
10	Creeping Day-flower	" [e]	LO–M	+
20	Lamb's Quarters	Rubbing[d]	LW–O	
53	Nightshade	" [d]	LW–O	
61	*Physalis angulata*	Aphid, rubbing	LD–M–Y–N–S	+
62	*Physalis lagascae*	Aphid	.....M–S–N	+
63	*Physalis sp.*	Aphid	LN–M–N	+
24	Pokeberry (Common)	Aphid, rubbing	LW–O	
25	Pokeberry (Southern)	" "	LW–M–S	+
86	Ragweed	" "	.....M–S–D–N	+
82	West Indian Gherkin	"	.....S–M–D	+
11	Wild Wandering Jew	" [e]	LO–M–Y	+

[a] These numbers refer to host species identically numbered in table 5.

[b] L refers to primary lesions: D-diffuse, N-necrotic, O-none developed, W-whitish, ...-uncertain of production. Other single letters refer to systemic symptoms: D-distortion, M-mottling, N-necrosis, O-no symptoms and no virus recovered, S-stunting, Y-yellowing.

[c] Leaf-rubbing methods failed to produce infection.

[d] Aphid inoculation methods failed to produce infection.

[e] Leaf-rubbing methods failed to produce infection but occasional infection was secured by hypodermic needle injection, stem and leaf mutilation methods with needle, and grafting methods.

It should be noted that the writer has studied, with some possible exceptions, the most common weeds that might obviously be hosts of the southern celery-mosaic virus occurring in the vegetable growing district around Sanford, Florida. There are undoubtedly many more susceptible wild hosts than those in table 3. Hosts that were tested, but gave negative results, are in the list that occurs on page 399, at the close of the discussion of experimental results. It is believed that 5 of the susceptible hosts are of sufficient interest to require individual description.

Wild Wandering Jew: Plants of the wild wandering jew, diseased with a virus, have been found widely distributed in nature (7) (8) (9) (11). They occur with great frequency in the weed patches along the edges of cultivated fields in the Sanford region and many other parts of Florida.

The spread of the celery virosis has been studied (11) in fields of this weed. The most important symptom is a mottling, which varies under some conditions from light green watered effects and mild mottling to irregular elliptical rings of bright yellow on the leaves. The chlorosis in some cases is so severe that it may involve a large proportion of the leaf. There is practically no distortion or stunting; generally the plant lives, flowers, and sets seed about as successfully when diseased as when free from the virus. Wild wandering jew was easily inoculated by aphids, but leaf-rubbing methods were uniformly unsuccessful.[2] No primary lesions developed, and the first symptom of infection was a light slightly water-soaked stripe that appeared in 10–18 days after inoculation. This striping became a vein-clearing pattern that, within a few days, changed into the mottling described.

Ragweed: Plants of ragweed with severe systemic mottling are usually easily found in the field (11). Such plants are somewhat stunted, the degree depending upon their ages when infected. The leaves tend to be small and marked with small yellow irregular spots. In some cases there is leaf distortion, and at times necrosis occurs. Under greenhouse conditions, ragweed seedlings have been infected by both aphid and leaf-rubbing methods of inoculation. No primary lesions have been observed, the first symptom being vein-clearing, which changed to yellow mottling. If plants are inoculated when growing under either unfavorably cool or dry conditions, or when in infertile soil, the bright mottled symptoms described are reduced to faint markings that verge on assuming a masked character. Diseased plants apparently were not much inhibited in flower and seed production.

Crane's-bill: Symptoms of virus infection in crane's-bill consist largely of stunting, mottling, and some distortion of the leaves. Inoculation with aphids resulted in producing disease, while leaf-rubbing methods gave negative results. No primary lesions were noted. Systemic symptoms were quite severe but did not inhibit seed production.

Physalis angulata: A wild Physalis (*P. angulata*) was frequently found in the field with a virus-diseased appearance. Seedlings were grown in the greenhouse and both aphid and leaf-rubbing methods of inoculation produced infection. Leaf rubbing resulted in the development of rounded yellowish primary lesions. In a few cases these affected leaves were dropped and the plant that bore them escaped infection. When aphids were used for inoculation, primary lesions and leaf drop affected the older leaves; but when the insects fed on the very young leaves as well, systemic

[2] While leaf-rubbing methods of inoculation have been unsuccessful in infecting *Commelina nudiflora*, it should be noted that needle inoculation in tips of stems, hypodermic injection methods, inarch or approach grafting methods, and tying of tips of bruised, healthy, and diseased tips together, have all been successfully used as means of obtaining virus transmission.

infection always occurred. The leaves of systemically infected plants were mottled, the plants were stunted, flowering and fruiting was reduced, and in some cases a dark streak type of necrosis occurred on the stems that resulted in dropping of leaves.

Southern Pokeberry: Pokeberry plants with brilliant systemic mottling of leaves were found in many different fields. All seedlings of this weed, grown in the greenhouse, were readily susceptible to primary infection with *Celery virus 1,* either through the use of aphids or leaf rubbing. In all cases primary lesions developed. These were midsize, round, whitish spots on inoculated leaves. Sometimes primary infected leaves were dropped and the virus did not become systemic. The remaining leaves of such individuals were, however, susceptible to infection, which apparently remained localized in the affected leaves. In a small number of cases systemic infection occurred, producing brilliantly mottled leaves but no severe reduction in growth of the plant, flower, or fruit production.

MISCELLANEOUS PLANTS SUSCEPTIBLE TO CELERY VIRUS

Certain plants that did not fall under the arbitrary classifications of vegetable crops, weeds, or ornamentals, were used in the study of the southern celery mosaic. Some of these hosts were convenient for use as material in the virus property studies (8), others were related to hosts already known to be susceptible, and symptoms on others, *e.g.,* banana varieties (10) and field corn, were studied as possible clues to relationships of viroses described by other workers on these hosts. Some of the results of these studies are reported in table 4, the rest of the data being in table 5.

The susceptibility of certain of the miscellaneous hosts was, it is believed, of special interest. Some have been, therefore, selected for short detailed descriptions.

Banana: Banana plants were inoculated by both aphid and leaf-rubbing methods. Infection was successful only when aphids were used to transmit the virus (10). No primary lesions were developed. The first symptoms of systemic infection occurred, after about a month's incubation period, on a leaf that was not visible at the time aphids were put on the plant to be inoculated. The first leaf to show symptoms was mottled along the veins. Later leaves were stunted, somewhat chlorotic, distorted, and marked with occasional black necrotic streaks. The leaf sheaths were arrested in their development, which caused a rosette of leaves and occasionally splitting of the pseudostem. Formation of the fruiting bud was inhibited in plants diseased at an early age.

Tobacco: A few seedlings of a broad-leaf type of tobacco affected with the celery virosis were found in a seedbed in the Sanford region. These plants were severely stunted and mottled and there was considerable leaf

TABLE 4.—*Results of studies on certain miscellaneous plants found susceptible to Celery virus 1 (compare also with Table 5 for species names, number of plants used, and recovery of virus)*

No.[a]	Miscellaneous plant	Inoculation method	Symptoms[b]	Occurrence in field
31	Broadbean	Rubbing	LP–O	
19	Buckwheat	"	.....M–S–D	
17	Cavendish Banana	Aphid	.....M–S–N–Y	
85	Chayote	Rubbing	LB–O	
4	Corn (U. S. D. A. strains)	Aphid[c]	LN–M–S–N–*S*	
72	*Datura meteloides*	Rubbing	LD–O	
3	Field Corn	Aphid[c]	LN–M–S–N–*S*	+
73	Jimson Weed	Rubbing	LD–O	
7	Kafir Corn	Aphid	.....M–*M*	
18	Lady Finger Banana	" [c]	LO–M–N–D–S–Y	+
6	Milo Maize	"	.....M–*M*	
64	Nicandra (Apple-of-Peru)	Rubbing	.....M	
68	*Nicotiana glutinosa*	Aphid, rubbing	LW–N–Y–M–S	
67	*Nicotiana sanderae*	Rubbing	.....M	
71	*Nicotiana sylvestris*	"	.....M	
60	*Physalis peruviana*	"	.....M	
9	Rye	Aphid[c]	.....M–Y	+
13	Spiderwort	"	.....Y–S–D–M	
5	Sweet Sorghum	"	.....M–S	+
65	Tabasco Pepper	Aphid, rubbing	LN–S–M–N	
1	Teosinté	Aphid[c]	.....M–Y–S	
69	Tobacco, Broadleaf	Aphid, rubbing	LN–M–D–N–S	+
70	Tobacco, Turkish	" "	LN–M–D–N–S	
8	Wheat	Aphid[c]	.....M–Y	
54	*Solanum aviculare*	Rubbing	LN	

[a] These numbers refer to host species identically numbered in table 5.

[b] L refers to primary lesions: P-purplish and small, B-black, D-diffuse, N-necrotic, O-none produced, W-whitish, ...-uncertain of production. Other single letters refer to systemic symptoms: D-distortion, M-mottling, *M-masking*, N-necrosis, O-no symptoms and no virus recovered, *S-striping*, S-stunting, Y-yellowing.

[c] Leaf-rubbing methods tried but failed to produce infection.

crumpling. Under greenhouse conditions, inoculations of plants of Broadleaf tobacco, Turkish tobacco, and *Nicotiana glutinosa* (*cf.* (8)) gave practically identical results. Infections were easily secured, whether by the use of either aphid or leaf-rubbing methods. Primary lesions occurred on plants infected by either method. They were not always visible, however, because of certain growing conditions during the incubation period. When primary lesions did develop they were whitish spots that became whiter for several days. In many cases some necrosis and collapse of tissues occurred as these symptoms advanced. In older leaves the non-necrotic whitish primary lesions started recovering and retaining their chlorophyll at about the time inoculated leaves began turning yellow from age. Symptoms of the first systemic infection usually were indicated by a brilliant vein clearing and crumpling of the young affected leaves. These symptoms changed

to a yellow and green mottling, and occasionally systemic necrosis developed. No diseased tobacco or *Nicotiana glutinosa* plants that were kept long enough, failed to bear seed capsules, although the fruits were fewer in numbers than from virus-free individuals.

Buckwheat: Plants of buckwheat were inoculated by the leaf-rubbing method and infected by the celery virus. The systemic mottling that developed on the leaves was very severe, accompanied by their malformation and a general stunting of the plants.

Wheat: Several varieties of wheat were grown and inoculated with aphid and leaf-rubbing methods. Aphid inoculations were alone successful in producing infection. No primary lesions were observed, and systemic symptoms of infection consisted of light and dark green mottling of leaves. No stunting was detected, nor were rosette types of symptoms noted.

Rye: Inoculation of rye seedlings with viruliferous aphids produced successful infection with the celery virus. Systemic symptoms were very similar to those noted on Commelina. Plants were not stunted and markings consisted largely at first of fine indistinct yellowish stripes of vein-clearing type, which soon turned to irregularly rounded and elliptical rings and spots.

Teosinté: Plants of teosinté were successfully infected with the celery virus by using aphids. No primary lesions were observed. When the virus had become systemic, the leaves had a generally yellowish cast due to a large number of small yellow spots. Diseased plants were small but not severely stunted, the leaves were chlorotic but not seriously yellowed, and the plants appeared to blossom at the same time as healthy individuals. Infected plants produced about as many seeds as did virus-free individuals.

Chayote: Leaves on several chayote vines were inoculated with celery virus by the leaf-rubbing method. Infection was nonsystemic and occurred as a few black, irregular, primary lesions.

Broadbean: Leaves on plants of broadbean produced small, dark, purplish primary lesions when inoculated with celery virus by the leaf-rubbing method. The lesions occurred on all plants tested, but apparently did not spread to produce systemic infection. The virus was not recovered from noninoculated leaves of the host.

COMPLETE EXPERIMENTAL HOST RANGE OF CELERY VIRUS 1

Table 5 presents in condensed form data from host-range studies that gave positive results. The susceptible hosts are arranged according to families. The numbers of plants inoculated and those infected are noted. Results of attempts at recovery of virus from leaves affected in different ways on the infected plants are also indicated. For complete data on symptoms produced by infection, for inoculation methods employed to pro-

TABLE 5.—*The complete experimental host range of Celery virus 1. These data are supplemented by other results, methods of inoculation, symptoms, and field occurrence, in the previous tables 1, 2, 3, and 4*

Family	Host names and number	Number of plants: Inoculated / Infected	Recovery of virus: Leaves tested	Recovery of virus: Result	Compare table
Gramineae	*Euchlaena mexicana* Schrad. (1—Teosinté)	18 / 16	Inoculated Mottled	+ +	4
	Zea mays L. (2—Maize, Sweet corn)[a]	210 / 120	Inoculated Striped	+ +	1
	Z. mays (3—Maize, Field corn)[a]	27 / 16	Inoculated Striped	+ +	4
	Z. mays (4—Corn, strains from U.S.D.A.)[a]	149 / 141	Inoculated Striped	+ +	4
	Holcus sorghum L. (5—Sweet sorghum)	29 / 13	Inoculated Mottled	..... +	4
	H. sorghum (6—Milo)	33 / 18	Inoculated Mottled	..... +	4
	H. sorghum (7—Kafir corn)	15 / 5	Inoculated Mottled	..... +	4
	Triticum aestivum L. (8—Wheat)[a]	70 / 42	Inoculated Mottled	+ +	4
	Secale cereale L. (9—Rye)	20 / 19	Inoculated Mottled	..... +	4
Commelinaceae[b]	*Commelina communis* L. (10—Creeping Day-flower)	20 / 20	Inoculated Mottled	+ +	3
	C. nudiflora L. (11—Wild Wandering Jew)	118 / 98	Inoculated Mottled	+ +	3
	Zebrina pendula Schnizl. (12—Wandering Jew)	16 / 16	Inoculated Mottled	0 +	2
	Tradescantia sp. (13—Spiderwort)	9 / 4	Inoculated Mottled	+ +	4
Liliaceae	*Lilium longiflorum* Thunb. (14—Easter Lily)	10 / 7	Inoculated Mild mottled	..... +	2
	Lilium auratum Lindl. (15—Golden Lily)	15 / 2	Inoculated Mottled	+ +	2
	Allium cepa L. (16—Onion)	60 / 15	Inoculated Yellowed	..... +	2
Musaceae	*Musa cavendishii* Lamb. (17—Cavendish banana)	2 / 2	Inoculated Mottled	0 0	4
	M. sapientum L. (18—Lady Finger banana)	15 / 13	Inoculated Mottled	0 0	4

TABLE 5.—(*Continued*)

Family	Host names and number	Number of plants Inoculated / Infected	Recovery of virus: Leaves tested	Recovery of virus: Result	Compare table
Polygonaceae	*Fagopyrum esculentum* Gaertn. (19—Buckwheat)	10 / 10	Inoculated Mottled	..... +	4
Chenopodiaceae	*Chenopodium murale* L. (20—Lamb's Quarters)	16 / 3	Inoculated Symptomless	+ 0	3
	Beta vulgaris L. (21—Red Garden beet)	25 / 25	Inoculated Mild mottled	+ +	1
	B. vulgaris cicla (22—Swiss Chard)	9 / 2	Inoculated Symptomless	+ 0	1
	Spinacia oleracea L. (23—Spinach)	43 / 38	Inoculated Yellowed	+ +	1
Phytolaccaceae	*Phytolacca americana (decandra)* L. (24—Pokeberry, common)	25 / 15	Inoculated Symptomless	+ 0	3
	P. rigida Small (25—Pokeberry, southern)	67 / 65	Inoculated Symptomless	+ 0	3
Aizoaceae	*Tetragonia expansa* L. (26—New Zealand Spinach)	14 / 4	Inoculated Symptomless	+ +	1
	Mesembryanthemum crystallinum L. (27—Iceplant)	10 / 10	Inoculated Malformed	+ +	2
Caryophyllaceae	*Lychnis viscaria* L. (28—German Catchfly)	5 / 5	.....	.....	2
Ranunculaceae	*Delphinium consolida* L. (29—Larkspur)	23 / 23	Inoculated Stunted	+ +	2
Cruciferae	*Brassica rapa* L. (30—Turnip, Purple Top White Globe)[c]	15 / 3	Inoculated Symptomless	+ 0	1
Leguminosae	*Vicia faba* L. (31—Broadbean)	10 / 10	Inoculated Symptomless	+ 0	4
Geraniaceae	*Geranium carolinianum* L. (32—Crane's-bill)	25 / 20	Inoculated Mottled	+ +	3
	Pelargonium hortorum Bailey (33—Common Bedding Geranium)	5 / 5	Inoculated Mottled	+ +	2
Tropaeolaceae	*Tropaeolum majus* L. (34—Nasturtium)	5 / 5	Inoculated Symptomless	+ 0	2
Umbelliferae	*Daucus carota* L. (35—Carrot)	40 / 31	Inoculated Mottled	0 +	1
	Anethum graveolens L. (36—Dill)	5 / 5	Inoculated Yellowed	..... +	1

TABLE 5.—(*Continued*)

Family	Host names and number	Number of plants Inoculated / Infected	Recovery of virus: Leaves tested	Recovery of virus: Result	Compare table
Umbelliferae	*Foeniculum vulgare* Hill (37—Fennel)	17 / 4	Inoculated Stunted	..... +	1
	Petroselinum hortense Hoffm. (38—Parsley)[a]	65 / 59	Inoculated Mottled	+ +	1
	Apium graveolens L. (39—Celery, Golden types)[a]	150 / 140	Inoculated Mottled	+ +	1
	A. graveolens L. (40—Celery, flavoring)	10 / 10	Inoculated Mottled	..... +	1
	A. graveolens L. (41—Celery, Green Pascal)	25 / 15	Inoculated Mottled	+ +	1
	A. graveolens rapaceum (42—Celeriac)	15 / 9	Inoculated Mottled	0 +	1
	Pastinaca sativa L. (43—Parsnip)	15 / 9	Inoculated Mottled	..... +	1
Apocynaceae	*Vinca rosea* L. (44—Madagascar Periwinkle)	35 / 35	Inoculated Mottled	+ +	2
Convolvulaceae	*Convolvulus sp.* (45—Bindweed)	15 / 12	.....	.....	3
	Ipomoea purpurea Lam. (46—Morning-glory)	6 / 3	Inoculated Mottled	+ +	2
	I. batatas Lam. (47—Sweet Potato)[a]	33 / 25	Inoculated Mottled	+ +	1
Polemoniaceae	*Phlox drummondii* Hook (48—Phlox)	20 / 20	Inoculated Symptomless	+ 0	2
	Gilia capitata Dougl. (49—Gilia)	12 / 6	Inoculated Mottled	0 +	2
Boraginaceae	*Cynoglossum amabile* Stapf & Drum. (50—Chinese Forget-me-not)	5 / 5	.....	.....	2
Hydrophyllaceae	*Phacelia whitlavia* Gray (51—California Bluebell)	15 / 12	Inoculated Mottled	0 +	2
	P. tanacetifolia Benth. (52—Phacelia)	13 / 13	Inoculated Necrotic	+ +	2
Solanaceae	*Solanum nigrum* L. (53—Nightshade)	42 / 40	Inoculated Symptomless	+ 0	3
	S. aviculare Forst. (54—*Solanum aviculare*)	12 / 6	.....	.....	4

TABLE 5.—(*Continued*)

Family	Host names and number	Number of plants Inoculated / Infected	Recovery of virus: Leaves tested	Recovery of virus: Result	Compare table
Solanaceae	*S. melongena* L. (55—Eggplant)	41 / 17	Inoculated Mottled	+ +	1
	Lycopersicon esculentum Mill. (56—Tomato)	45 / 10	Inoculated Mottled	0 +	1
	Atropa belladonna L. (57—Belladonna)	5 / 5	.....	.....	2
	Physalis alkekengi L. (58—Strawberry groundcherry)	5 / 5	Inoculated Mottled	+ +	1
	P. pubescens L. (59—Common groundcherry)	12 / 12	Inoculated Mottled	+ +	1
	P. peruviana L. (60—*Physalis peruviana*)	9 / 9	Inoculated Mottled	..... +	4
	P. angulata L. (61—*Physalis angulata*)	29 / 19	Inoculated Mottled	+ +	3
	P. lagascae R. & S. (62—*Physalis lagascae*)	14 / 12	Inoculated Mottled	+ +	3
	P. sp. (63—*Physalis sp.*)	6 / 6	Inoculated Mottled	+ +	3
	Nicandra physalodes Pers. (64—Nicandra, Apple-of-Peru)	5 / 5	.....	.....	4
	Capsicum annuum L. (65—Tabasco Pepper)	31 / 31	Inoculated Mottled	+ +	4
	C. frutescens L. (66—Sweet Pepper)[a]	32 / 28	Inoculated Mottled	+ +	1
	Nicotiana sanderae Sander. (67—Nicotiana)	14 / 7	.....	.....	4
	N. glutinosa L. (68—*Nicotiana glutinosa*)	24 / 24	Inoculated Mottled	+ +	4
	N. tabacum L. (69—Broadleaf tobacco)	57 / 57	Inoculated Mottled	+ +	4
	N. tabacum L. (70—Turkish tobacco)	25 / 25	Inoculated Mottled	+ +	4
	N. sylvestris Spegaz. & Comes. (71—*Nicotiana sylvestris*)	5 / 5	Inoculated Mottled	..... +	4
	Datura meteloides DC. (72—*Datura meteloides*)	4 / 4	Inoculated Symptomless	+ 0	4

TABLE 5.—(*Continued*)

Family	Host names and number	Number of plants: Inoculated / Infected	Recovery of virus: Leaves tested	Recovery of virus: Result	Compare table
Solanaceae	*D. stramonium* L. (73—Jimson weed)	9 / 9	Inoculated Symptomless	+ 0	4
	Petunia hybrida Vilm. (74—Petunia)[a]	30 / 30	Inoculated Mottled	+ +	2
Scrophulariaceae	*Antirrhinum majus* L. (75—Snapdragon)	16 / 16	Inoculated Chlorotic	+ +	2
Dipsaceae	*Scabiosa atropurpurea* L. (76—Sweet Scabiosa)	5 / 5	Inoculated Mottled	+ +	2
Cucurbitaceae	*Cucurbita pepo* L. (77—Pumpkin)	12 / 5	Inoculated Mottled	..... +	1
	C. pepo condensa (78—Golden Summer Crookneck squash)	40 / 39	Inoculated Mottled	+ +	1
	C. pepo condensa (79—Cocozelle squash)	40 / 39	Inoculated Mottled	+ +	1
	C. maxima Duchesne (80—Hubbard squash)	5 / 5	Inoculated Mottled	+ 0	1
	Citrullus vulgaris Schrad. (81—Watermelon)[a]	37 / 31	Inoculated Mottled	+ +	1
	Cucumis anguria L. (82—West Indian Gherkin)	5 / 5	Inoculated Mottled	..... +	3
	C. sativus L. (83—Cucumber)[a]	100 / 100	Inoculated Mottled	+ +	1
	C. melo L. (84—Muskmelon)[a]	20 / 17	Inoculated Mottled	+ +	1
	Chayota edulis Jacq. (85—Chayote)	7 / 3	Inoculated Symptomless	+ 0	4
Compositae	*Ambrosia elatior* (*artemisiifolia*) L. (86—Ragweed)	75 / 62	Inoculated Mottled	..... +	3
	Zinnia elegans Jacq. (87—Zinnia)	10 / 10	Inoculated Mottled	..... +	2
	Tagetes patula L. (88—French Marigold)	5 / 5	Inoculated Mottled	..... +	2
	T. erecta L. (89—Aztec Marigold)	5 / 5	Inoculated Mottled	..... +	2
	Emilia sagittata (Vahl.) DC. (90—Floras Paintbrush)	20 / 20	Inoculated Symptomless	+ +	2

[a] Several varieties used.

[b] Since completing these trials further study was made on *Commelina erecta* L., which up until preparation of this manuscript appeared to be immune to celery virus infection. Of 47 plants 2 were diseased when inoculated by aphids, virus was recovered; no infected plants have ever been seen in the field.

[c] The author secured these data from one trial on turnips in Florida. Numerous other attempts on the same variety and several others failed to secure infection. These latter trials extended for over 2 years (see Table 1, footnote d, and the list of negatively reacting hosts on page 399).

duce infection, and for occurrence of the disease in the field, see tables 1, 2, 3, and 4, as indicated in the accompanying table.

In many cases many more plants than are listed in table 5 were used in inoculation studies. The preliminary trials, and other unsuccessful attempts performed to ascertain the best methods and ages of plants to be inoculated are not included in the table. Examples of certain of these numbers of plants not included in the table may be cited: over 200 corn seedlings used in studying possible effects of leaf-rubbing methods; about 150 tomato plants inoculated at different times on cotyledons; 112 plants of *Commelina nudiflora* inoculated with hypodermic needle, leaf-rubbing and mutilation methods; 36 onion plants grown from sets inoculated in every conceivable manner; many annual ornamentals inoculated at a very young stage; nearly 1000 plants of numerous species that were growing poorly and were inoculated during cold cloudy spells; and several series of plants of various species, discarded because accidental infection occurred in untouched as well as inoculated individuals.

HOSTS THAT GAVE NEGATIVE REACTION TO CELERY-VIRUS INOCULATIONS[3]

Polypodium vulgare L. (Polypody fern), *Adiantum capillus-veneris* L. (Southern maidenhair fern), *Typha angustifolia* L. (Cat-tail), *Saccharum officinarum* L. (Sugar cane), *Stenotaphrum secundatum* Kuntze (St. Augustine grass), *Panicum barbinode* Trin. (Para grass), *Tricholaena rosea* Nees (Natal grass), *Oryza sativa* L. (Rice), *Avena sativa* L. (Oat)[a], *Cynodon dactylon* Pers. (Bermuda grass), *Hordeum vulgare* L. (Barley)[a], *Poa pratensis* L. (Kentucky Blue grass), *Cyperus compressus* L., *Cyperus esculentus* L. (Chufa), *Xanthosoma sp.* (Elephant's-ear), *Pontederia cordata* L. (Pickerelweed), *Piaropus crassipes* (Mart.) Britton (Common Water hyacinth), *Dendropogon usneoides* (L.) Raf. (Spanish moss), *Ananas sativas* Schult. (Pineapple), *Tillandsia fasciculata* Sw. (Air plant), *Commelina erecta* L.[b], *Tulipa gesneriana* L. (Tulip), *Asparagus officinalis* L. (Asparagus), *A. plumosus* Baker (Asparagus fern), *Sansevieria thrysiflora* Thunb. (Bowstring hemp), *Hemerocallis flava* L. (Yellow day lily), *Yucca gloriosa* L. (Spanish dagger), *Hippeastrum vittatum* Herb. (Amaryllis), *Hymenocallis* sp. (Spider lily), *Narcissus tazetta* L. (Narcissus), *Dioscorea bulbifera* L. (Air potato), *Gladiolus lemoinei* Hort. (Gladiolus), *Canna glauca* L. (Canna), *Chenopodium botrys* L. (Jerusalem oak), *Kochia scoparia* Schrad. (Summer cypress), *Amaranthus spinosus* L. (Spiny pig-

[3] Throughout this section the lower-case superior letters after the names of plants can be interpreted as follows:

[a] A number of different named varieties tested.

[b] See footnote b in table 5.

[c] See footnote d in table 1, and footnote c in table 5.

[d] Tender seedlings tested while succulent.

weed), *A. hybridus* L. (Princes feather), *Celosia argentia* L. (Cockscomb), *Gomphrena globosa* L. (Globe amaranth), *Mirabilis jalapa* L. (Four o'clock), *Portulaca grandiflora* Hook (Rose moss), *Alsine media* L. (Chickweed), *Dianthus plumarius* L. (Grass pink), *D. caryophyllus* L. (Carnation), *Gypsophila paniculata* L. (Babies'-breath), *Nigella damascena* L. (Love-in-a-mist), *Papaver somniferum* L. (Opium poppy), *P. orientale* L. (Oriental poppy), *P. rhoeas* L. (Shirley poppy), *Brassica oleracea* L. *acephala* (Kale), *B. oleracea gemmifera* (Brussels sprouts), *B. oleracea capitata* (Red cabbage),[a] *B. oleracea capitata* (Green cabbage),[a] *B. oleracea capitata* (Savoy cabbage), *B. olercea botrytis* (Cauliflower),[a] *B. oleracea botrytis* (Broccoli),[a] *B. caulorapa* Pasq. (Kohlrabi), *B. napus* L. (Rape), *B. napobrassicae* Mill. (Rutabaga), *B. rapa* L. (Turnip),[a, c] *B. pekinensis* Rupr. (Chinese cabbage, Petsai), *B. nigra* Koch (Black mustard), *B. alba* Rabenh. (White mustard), *Lepidium virginicum* L. (Peppergrass), *Mathiola incana* R. Br. (Stock), *Lobularia maritima* Desv. (Sweet alyssum), *Iberis umbellata* L. (Candytuft), *Raphanus sativus* L. (Radish),[a] *Reseda odorata* L. (Mignonette), *Rosa dilecta* Rehd. (Radiance rose), *R. laevigata* Michx. (Cherokee rose), *Pisum sativum* L. (Garden pea),[a] *Lathyrus odoratus* L. (Sweet pea),[a] *Cicer arietinum* L. (Chickpea, Garbanzo), *Arachis hypogaea* L. (Peanut), *Phaseolus vulgaris* L. (Garden bean),[a] *P. limensis* Macf. (Lima bean), *Chapmania floridana* T. & G. (Bush clover), *Vigna sinensis* Endl. (Cowpea),[a] *Dolichos lablab* L. (Hyacinth bean), *Clitoria ternatea* L. (Butterfly pea), *Crotalaria spectabilis* Roth. (Rattlebox), *Crotalaria sp.*, *Glycine max* Merr. (Soybean),[a] *Trifolium repens* L. (White clover), *Lupinus hirsutus* L. (European blue lupine), *Ionoxalis violacea* (L.) Small (Violet wood-sorrel), *Xanthoxalis corniculata* (L.) Small (Yellow Wood-sorrel), *Citrus grandis* Osbeck (Grapefruit),[d] *C. aurantium* L. (Sour orange),[d] *C. sinensis* Osbeck (Orange),[d] *C. nobilis* Lour. (Tangerine),[d] *Euphorbia pulcherrima* Willd. (Poinsettia), *E. marginata* Pursh. (Snow-on-the-mountain), *Codiaeum variegatum* Blume (Croton), *Ricinus communis* L. (Castor bean), *Manihot ultissima* Pohl. (Cassava), *Impatiens balsamina* L. (Balsam), *Gossypium hirsutum* L. (Upland cotton), *Hibiscus esculentus* L. (Okra),[a] *H. rosa-sinensis* L. (Chinese hibiscus), *Hibiscus Viola floridana* Brainerd (Florida violet), *V. odorata* L. (English violet), *V. primulifolia* L. (White violet), *V. tricolor* L. (Pansy), *Carica papaya* L. (Papaya),[d] *Psidium guajava* Raddi. (Guava),[d] *Clarkia elegans* Dougl. (Clarkia), *Trachymene caerulea* R. Graham (Laceflower), *Ferula communis* L. (Giant fennel), *Limonium latifolium* Kuntze (Statice), *Anchusa officinalis* L. (Alkanet), *Myosotis scorpioides* L. (True Forget-me-not), *Heliotropium peruvianum* L. (Common heliotrope), *Verbena hybrida* Voss. (Verbena), *Lantana sellowiana* Link & Otto (Weeping lantana), *Salvia splendens* Ker. (Scarlet sage), *Mentha spicata* L. (Spearmint), *Solanum*

tuberosum L. (Potato),[a] *S. pseudo-capsicum* L. (Jerusalem cherry), *Salpiglossis sinuata* Ruiz & Pav. (Scalloped salpiglossis), *Schizanthus pinnatus* Ruiz & Pav. (Wingleaf Butterfly flower), *Digitalis purpurea* L. (Foxglove), *Linaria canadensis* (L.) Dumort (Toadflax), *L. floridana* Chapm., *Gerardia divaricata* Chapm. (False foxglove), *Plantago sp.* (Plantain), *Campanula medium* L. (Canterbury bells), *Lobelia erinus* L. (Lobelia), *Sonchus oleraceus* L. (Sow thistle), *Cichorium intybus* L. (Chicory),[a] *C. endivia* L. (Endive),[a] *Tragopogon porrifolius* L. (Salsify), *Lactuca sativa L. crispa* (Curled lettuce),[a] *L. sativa capitata* (Head lettuce),[a] *L. sativa longifolia* (Romaine lettuce), *Achillea millefolium* L. (Milfoil), *Chrysanthemum carinatum* L. (Annual chrysanthemum), *C. morifolium* Ram. (Florist's chrysanthemum), *C. parthenium* Pers. (Feverfew), *C. leucanthemum* L. (Ox-eye daisy), *Helianthus annuus* L. (Sunflower), *Bidens leucantha* (L.) Willd. (Spanish needle), *Cosmos bipinnatus* Cav. (Cosmos), *Dahlia pinnata* Cav. (Dahlia), *Coreopsis tinctoria* Nutt. (Calliopsis), *C. drummondii* Torr. & Gray (Goldenwave), *Callistephus chinensis* Nees. (China aster),[a] *Aster novae-angliae* L. (New England aster), *Erigeron sp.*, *Gaillardia pulchella* Foug. (Gaillardia), *Calendula officinalis* L. (Pot marigold), *Senecio cruentus* DC. (Cineraria), *Helichrysum bracteatum* Andr. (Strawflower), *Ageratum houstonianum* Mill. (Ageratum), *Centaurea cyanus* L. (Cornflower).

DISCUSSION

The host range of *Celery virus 1* as herein reported is not claimed to be complete. Other means of inoculation than those tried, applied to plants under varying conditions, might result in infection on hosts now considered immune from celery virosis. Both the aphid and leaf-rubbing methods of inoculation were very severe, and the basis of the selection of a number of the hosts used in the studies was their apparent occurrence as naturally diseased plants in the field. About one third of the hosts tested for susceptibility to *Celery virus 1* were successfully infected with the virus.

The celery virus was found to infect 4 monocotyledonous and 19 dicotyledonous families. The writer does not consider such a wide host range as being characteristic of *Celery virus 1* alone; indeed, it is possible that other viruses now believed to have a narrow host range, may, when studied under more variable conditions and with more variable technique, prove to have a host range as wide or wider than that described for the southern celery-mosaic virus. Grant (3) has recently extended the host range of ordinary tobacco-mosaic virus beyond a point that, a few years ago, would have been considered an impossibility.

With some viruses, *e.g.*, ring spot (12) and aster yellows (6), symptoms on a fairly wide range of hosts have many characteristics in common. Holmes (4) has shown that the effect of a single strain of a virus, ordinary

tobacco virus, on members of a single family of plants, the Solanaceae, may have great variableness in symptoms produced. In the present studies of *Celery virus 1* the extremes in systemic symptom expression have been found of remarkable range. So different are these symptoms on various hosts that in some cases, unless the whole history of the experiments were known, it would be hard to believe they were all produced by the same causative agent.

In some cases the development of primary lesions was dependent on leaf-rubbing inoculation, as in lamb's quarters, while in maize primary lesions did not occur unless aphids were used for inoculation purposes. In many other instances both methods of inoculation produced primary lesions. These lesions varied widely in character: Large black necrotic spots developed that involved the whole leaf, as in squash; diffuse lesions were produced on Datura, in some cases lesions appeared before systemic symptoms were evident, as in cucumber; in other instances lesions appeared after the systemic symptoms, as in tomato; there was the production of whitish lesions on the Phytolaccas, and the development of whitish lesions in phlox, which later recovered their chlorophyll and retained it when the rest of the leaf became yellow from age.

The symptoms of systemic infection were even more various than the phenomena exhibited by the symptoms of primary lesions. As examples may be cited the following: extreme blight and death of spinach, severe stunt of larkspur, stunt and mottle of Cocozelle squash, brilliant mottle and brown streak-like necrosis of celery, black necrosis and stunt of watermelon, severe stunt and rosette of banana, stunt and stripe of maize, medium mottle of tobacco, mild mottle and severe yellowing of snapdragon, mild mottle and severe stunt of tomato, brilliant yellow markings but very little stunting in Commelinas, escape of systemic infection by dropping of diseased leaves as in Datura, occasional masked symptoms of eggplant, and apparently consistent masking of symptoms in Floras paintbrush.

It is of special importance to vegetable growers that, with the exception of a few plants in one series of one variety of turnips, repeated attempts to infect as many members of the mustard and cabbage family as could be secured have failed to produce infection with *Celery virus 1*. The lettuce, endive, and chicory varieties also remained free from infection. Among the leguminous vegetable crops many varieties of garden beans, peas, soybeans, and field or cowpeas did not succumb to the virus. Irish potatoes were not susceptible to infection.

Varieties of rice, oats, and barley were tested for susceptibility to *Celery virus 1* but remained unaffected. A number of pasture and other grasses also remained free of infection.

It is well to understand that in many cases of susceptible hosts reported

in this paper apparent immunity may exist in the field. Under experimental conditions it is possible to force infection of certain hosts by the virus, either by confining aphids that would not ordinarily occur on them, or by the leaf-rubbing method, which may be a much more drastic means of transmittal than that occurring in nature. It is because of such reasons that data on natural field occurrence of diseased plants were particularly sought and recorded.

SUMMARY

The southern celery-mosaic virus (*Celery virus 1*) has been reported by the writer from certain portions of the subtropical United States (Florida), from the tropics (Cuba), and it is probable that it occurs in other subtropical and tropical regions. Results reported in this paper are largely from studies made in Florida.

Inoculation experiments, supplemented by symptom studies and field observations, have demonstrated that the southern celery-mosaic virus may infect at least 91 different host plants, comprising 4 families of the monocotyledonae and 19 families of the dicotyledonae. Of the susceptible host plants, 46 have been found naturally infected in the field. In addition to the 91 susceptible hosts mentioned, 146 species were tested and were apparently not susceptible to infection by *Celery virus 1*.

Some of the important 29 susceptible vegetable crops are: different types of celery, sweet corn, cucumber, ground cherry, sweet pepper, spinach, squash, and sweet potato. Some important vegetable crops that did not succumb to the virus infection are: different types of cabbage and mustard, garden and field beans, garden pea, lettuce, endive, and Irish potato.

Some of the important 23 ornamental hosts are: Aztec and French marigolds, Floras paintbrush, geranium, larkspur, periwinkle, petunia, and zinnia. Some of the important 14 susceptible weed hosts are: Crane's-bill, species of Physalis, pokeberrys, ragweed, wild wandering jew, and creeping day-flower. Some of the important 25 susceptible miscellaneous plants are: broadbean, bananas, varieties of sorghum, tobacco and other solanaceous species, wheat, and rye.

Extreme variability in symptoms was notable. Primary lesions resulted in certain cases from both aphid and rubbing methods of inoculation and systemic infection did not always follow primary-lesion development. Severe necrosis, stunting, yellowing, mottling, and distortion developed on some hosts, while in certain others these symptoms were mild, in some cases infected leaves were dropped, which precluded systemic disease, and masked carriers were noted in certain species of plants.

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WOJNOWICIA GRAMINIS AS A VERY WEAK, SECONDARY PARASITE OF WINTER CEREAL CROPS[1]

RODERICK SPRAGUE[2]

(Accepted for publication December 8, 1934)

INTRODUCTION

For a number of years after Heald (7) first reported finding the prairie foot rot of winter wheat near Spokane, Washington, two fungi that did not sporulate in culture were regularly isolated from the soil-line lesions of diseased wheat and barley culms[3] growing on certain high prairies in the Columbia Basin of Oregon and Washington. One of these fungi was identified by McKinney and Johnson (14) and McKinney (13) as *Wojnowicia graminis* (McAlp.) Sacc. and D. Sacc and the other by the writer as *Cercosporella herpotrichoides* Fron (21). In another article, the writer (18) briefly mentioned that, under experimental inoculation conditions, *Wojnowicia graminis* was a very weak parasite on winter wheat in Oregon, and all his studies conducted since have confirmed the original findings, namely, that the Cercosporella foot rot (Columbia Basin foot rot) is due to *Cercosporella herpotrichoides* and that *W. graminis* is a very weak secondary parasite.

DISTRIBUTION

Wojnowicia graminis is wide spread in Australia (3, 12, 15, 16), in the Pacific Northwest, and throughout the Great Plains area of North America. Kirby (10) reported *W. graminis* associated with *Ophiobolus graminis* Sacc. in grain in New York. Foëx (4), Foëx and Rosella (5, 6), and Kerleroux (9) found it associated with several fungi near Paris, France, and van de Laar (11) reported it from Holland. Probably it occurs in favorable localities throughout northwestern Europe.

Approximately 125 isolations of *Wojnowicia graminis* mentioned by Sprague and Fellows (22) were obtained from Cercosporella-infected culms of wheat and barley grown in various prairie areas in Wasco and Union

[1] Cooperative investigations between the Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture, and the Oregon and Washington Agricultural Experiment Stations with field headquarters at Corvallis, Oregon. Published as Technical Paper No. 229 of the Oregon Technical Series.

[2] Assistant pathologist, Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture. The writer acknowledges the valued assistance of Dr. A. G. Johnson in preparing the manuscript for publication.

[3] Isolations made by Dr. Hurley Fellows during the years from 1923 to 1929 from wheat collected in Union County, Oregon, consisted predominantly of these two fungi. (Unpublished reports.)

Counties, Oregon, and Klickitat County, Washington. As reported elsewhere (19), *Cercosporella herpotrichoides* in the Pacific Northwest is restricted to sod-grass-prairie areas in foothills in the Columbia Basin where the average annual precipitation ranges from 15 to 25 inches. However, the range of *W. graminis* extends to some extent into semiarid portions of Wasco and Umatilla Counties, Oreg., and Asotin, Walla Walla, Columbia, Spokane, Whitman, and Klickitat Counties, Wash. In Asotin County it is abundant in the roots and crowns of Turkey wheat in fields several miles from the Cercosporella-infested area near Anatone. In Turkey wheat it is associated with a condition resembling the Helminthosporium foot-rot complex in the Great Plains area. *W. graminis* apparently thrives in the semiarid regions, where the average annual precipitation may range from as low as 12 inches to approximately 25 inches. Nearly all of the isolations have been made from wheat plants grown in various phases of fine or very fine sandy loam soils. *W. graminis* is associated with *C. herpotrichoides* in the basal culms of wheat growing in a mellow, dark soil near Anatone, Wash., which, according to Dr. W. L. Powers of the Oregon Experiment Station, is essentially the same as the black soil of Russia. In Walla Walla County *W. graminis* was isolated from almost 100 per cent of the culms of Hybrid 128 wheat, a winter variety, growing in Walla Walla series fine sandy loam in certain low swales. The fungus has not been found to date in the acid soils of humid western Oregon nor does it occur very regularly in heavy soils in the semiarid portions of the Pacific Northwest.

SYMPTOMS

Carne (3) states that *Wojnowicia graminis* causes a root rot on the wheat plant and produces partly superficial, dark brown to black spots and streaks on the culm. He says that the fungus seldom appears above ground on the outside of the culms. Foëx (4) states that *W. graminis* was isolated twice in the spring of the year at Versailles from wheat plants with superficial brown, circular spots extending from the second internode towards the lower internode.

In the Pacific Northwest the action of *Wojnowicia graminis* has been so closely associated with that of *Cercosporella herpotrichoides* that very few specific symptoms are recognizable. Besides this association with the recognized symptoms caused by *C. herpotrichoides, W. graminis* is associated with slight etching or superficial streaking of the basal parts of the culms and also to some extent with a certain type of browning of roots close to the crown. It also invades the central cavity of nearly ripe culms and causes them to take on a lead color for several inches above the ground. Sometimes, in late season, this condition extends beyond the zone attacked by *C. herpotrichoides* (Fig. 1). In Walla Walla County and north into

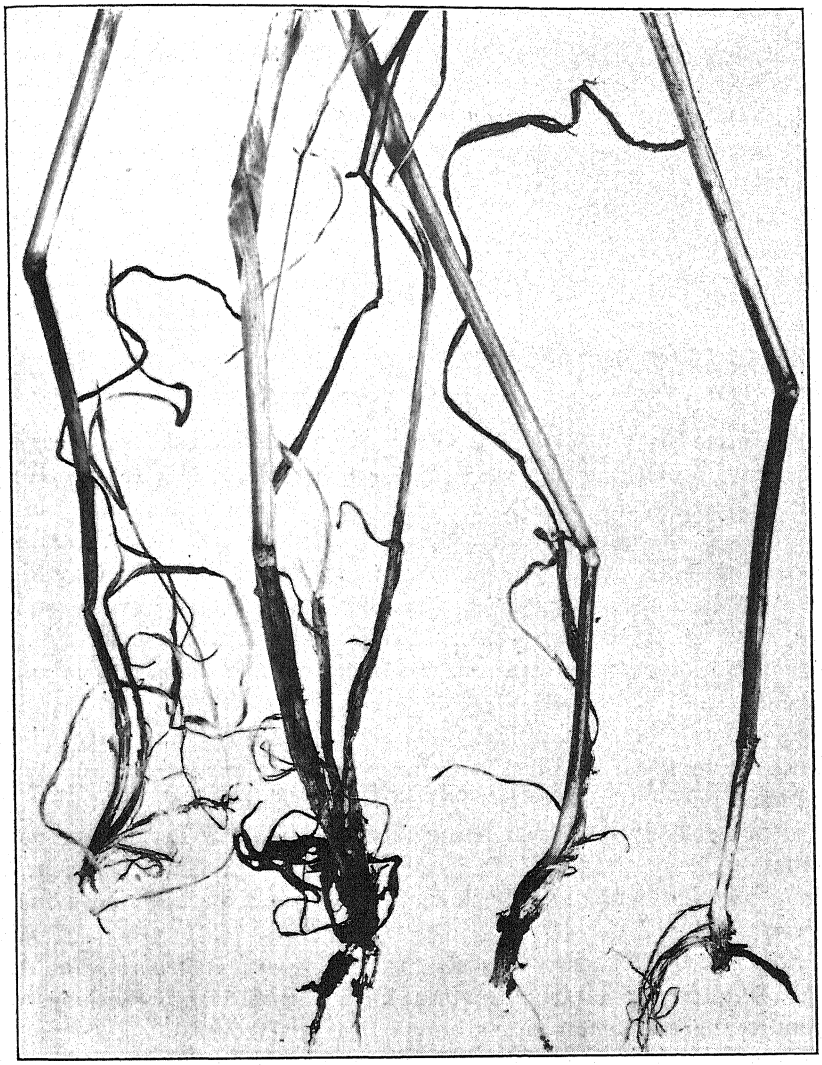


FIG. 1. Winter rye, in advanced soft-dough stage, from field near Imbler, Ore., showing Cercosporella lesions near soil line and extension of discoloration of culm for several inches above through action of *Wojnowicia graminis* after a heavy rain in late June, 1931. $\times \frac{2}{3}$.

Spokane County, Wash., *W. graminis* has been found to invade the central cavity of the culms of tall, nearly ripe wheat that did not show symptoms of Cercosporella foot rot.

HOST RANGE

Wojnowicia graminis has been isolated from the following fall-sown grains and grasses grown under natural conditions in the field: Winter

wheat, *Triticum vulgare;* emmer, *T. dicoccum;* einkorn, *T. monococcum;* spelt, *T. spelta;* barley, *Hordeum vulgare;* rye, *Secale cereale;* winter oats, *Avena sativa;* bunch grass, *Agropyron inerme* and *A. riparium;* and cheat grass, *Bromus tectorum; Koeleria cristata;* and *Poa sandbergii.* All of the grass hosts were found in one location in Spokane County, Wash., except *B. tectorum,* and in all these cases of grass hosts the fungus was associated with *Cercosporella herpotrichoides. Wojnowicia graminis* does not occur commonly on oats and einkorn. Pycnidia of *W. graminis* were found only on winter wheat. On all the other hosts the fungus was isolated and identified by the vegetative mycelium. No attempt was made to study the fungus in the spring-sown grains.

INOCULATION STUDIES

While the foot rot ascribed to *Wojnowicia graminis* has been recognized as a serious problem in Australia for many years (3, 12, 15, 16) apparently no critical study was made to determine the relative pathogenicity of this and other fungi until recently. Correspondence with W. V. Ludbrook, Division of Plant Industry, at Canberra City, Australia, who very kindly furnished the writer two representative pure cultures of *W. graminis,* indicates, however, that this neglected phase is being studied and that the former supposition that the organism was highly pathogenic is now in considerable doubt.

McKinney (13) stated that *Wojnowicia graminis* was only slightly pathogenic to wheat seedlings and suggested further studies. Heald (8) mentioned inoculating winter wheat with pure cultures of the fungus obtained both locally and from Hurley Fellows. The writer, as an undergraduate student, assisted F. D. Heald at the time in this experiment and recalls that the results at best indicated only weak pathogenicity for *W. graminis.*

Broadfoot (2) recently reported that *Wojnowicia graminis* obtained from 6 localities in Alberta Province did not cause seedling injury. His studies were conducted as pot cultures in the greenhouse.

In 1929 germinating wheat was grown at Corvallis on sterile quartz sand saturated with nutrient broth in bottles and on potato dextrose agar in flasks, and inoculated with several strains of *Wojnowicia graminis.* One group was incubated at 15° C. and another group at 20° C. In the quartz sand there were 3 cases out of 8 at 15° C., where the fungus invaded through the edge of the leaf sheath for a short distance at points where the sheath came in contact with the spreading fungus colony. The lesions failed to spread beyond the initial development. The wheat in all other bottles and flasks, including all uninoculated controls, remained healthy.

In the field, on Tygh Ridge, Oreg., 16-foot rows of Hybrid 128 wheat,

sown August 29, were inoculated in duplicate with 3 separate strains of *Wojnowicia graminis* isolated from Cercosporella-infested wheat from Klickitat County, Wash., and Union County, Oreg. The wheat on Tygh Ridge, which was in a noninfested field, free of any natural infestation with *Cercosporella herpotrichoides,* and 2 miles from the nearest infestation, was commercially seeded in early September and inoculated with *W. graminis* on October 10, 1929. Viable inoculum, which was grown in liter flasks on sterile seed of oats and barley, was applied at the base of the crowns and covered with an inch of moist soil. The inoculum was applied at approximately the rate of ½ liter per rod row. Additional inoculum was scattered over the plants. In April, 1930, the outer leaf sheaths of the inoculated plants were brown and decayed and pycnidia of *Wojnowicia graminis* were protruding from the sheaths. The injury was, however, only one sheath layer deep and the plants were otherwise as normal as the uninoculated plants that surrounded the inoculated portion. This experiment on Tygh ridge was in the same field with that reported earlier (21) for Cercosporella in which the wheat inoculated with *C. herpotrichoides* under the same conditions was severely injured.

Essentially the same type of experiment was continued on High Prairie, Klickitat County, Wash., in the winter and spring of 1930–31 with the same results, except that *Wojnowicia graminis* failed to produce pycnidia. There were faint etchings on the culm of the inoculated plants, but isolations yielded only *Fusarium culmorum* and *Fusarium* spp. but not *W. graminis.*

In the field at Corvallis, Oreg., duplicated rod rows of Huston, Holland, and Purplestraw wheat, growing in Newberg sandy loam, were inoculated on December 23 at seeding time (dry fall prevented earlier seeding) as in the above reported experiments. There was very slight injury to a few crowns but the injury was negligible as compared to the destruction wrought by *Cercosporella herpotrichoides* in a nearby plot under the same inoculation conditions (21).

In the greenhouse at Corvallis miscellaneous inoculation series have been conducted from time to time during the 6-year period from 1929 to 1934. The conditions in the greenhouse were as follows: No heat was used and the humidity was kept as high as possible, which, during the mild, moist winters, that prevail at Corvallis, gave nearly ideal growing conditions for most cereal foot-rot fungi that were being studied. At first the inoculated plants were grown in deep flats but, since 1929, they have been grown either in soil in the bench or in cubical fir boxes that have a capacity of approximately one cubic foot. Newberg fine sandy loam was used unsterilized, as it was free from *Wojnowicia graminis.* Most of the inoculations were made on club hybrid wheats (Hybrid Nos. 63, 128, or 143) at seeding time or

several weeks after emergence. In addition, inoculations were made on white winter barley, rye, *Agropyron spicatum, Festuca ovina, Agropyron tenerum, A. cristatum,* and *Bromus rigidus* with practically negative results. These experiments totalled about 3,000 inoculated culms. No exact culm or plant counts were made on some of the experiments. In all of them the usual etchings and occasional saprophytic stromatic growth were noted in a small percentage of the culms, the rest being apparently healthy. Positive isolations were obtained from some of the wheat culms that showed superficial injury.

The results of inoculation with the two Australian strains and one Oregon strain (J. J. I.) are given in table 1. Reisolations were attempted from representative lesions of all hosts and were positive only in 2 out of 10 from wheat, 6 out of 10 from rye, and 13 out of 15 from barley. The results indicated, as in previous studies, that *Wojnowicia graminis* does little damage to most cereals. In the case of white winter barley the total weight of the 4-foot row inoculated with Australian culture No. 8/248 was 23.2 grams and the uninoculated check 58.5 grams. However, in 10 of the 16 rows inoculated with the Australian cultures, the yields from the uninoculated rows weighed more than those from the controls, and the grand total yield for the 16 inoculated rows was 1,556.7 grams, and that for the inoculated ones was 1,516.4 grams. Apparently, therefore, *W. graminis* is almost of no economic importance, except possibly on barley and rye.

TAXONOMY

The genus Wojnowicia Sacc. (17) differs mainly from Hendersonia Berk. in having blunt setae or hairs present on the pycnidium. The long, curved, spindle-form spores described for the several species of Wojnowicia on Gramineae have their counterpart in several nongraminicolous species of Hendersonia, such as *H. peponis* Rolland or *H. fusariodes* Sacc. as illustrated by Allescher (1). While it is apparent that the genus Hendersonia, as now interpreted, needs critical study, the spores of Wojnowicia fall within the limits of Hendersonia, and, in final analysis, the hairs on the pycnidium of Wojnowicia constitute the only difference between the two genera. The writer questions the original necessity of erecting the genus Wojnowicia, but he prefers to retain for the present the now recognized name of *W. graminis.* The writer wishes to point out further that the described species of Wojnowicia and Hendersonia on Gramineae should be studied to determine synonomy. He suspects, but is not in position to obtain type materials, that most of the species of these genera described on grasses and cereals belong to a single, somewhat variable species.

Table 2 lists a number of these suspects with the spore measurements of the types given where known.

TABLE 1.—*Result of inoculating in the greenhouse various grains and grasses seeded October 19, 1933, with wheat-grain cultures of Wojnowicia graminis. Inoculated December 18, 1933. Plants dug and results recorded at or near heading time March 27, 1934*

Grain or grass inoculated	Number of plants with or without lesions											
	Inoculated with Oregon culture		Not inoculated		Inoculated with Australian culture 211		Not inoculated		Inoculated with Australian culture 8/248		Not inoculated	
	Lesions slight	Healthy	Lesions slight	Healthy	Lesions slight	Healthy	Lesions slight	Healthy	Lesions slight	Healthy	Lesions slight	Healthy
Fulhio wheat	0	71	0	80	0	43	0	57	0	81	0	54
Holland wheat	4	65	3	68	6	32	3	27	8	24	0	31
Huston wheat	0	69	0	81	3	41	0	61	0	39	0	47
Golden wheat	0	70	0	66								
White Winter barley	2	99	0	105	24	71	7	70	45	47	0	89
Winter rye	18	67	0	86	5	31	6	36	0	38	0	19
Bearded Winter spelt	2	91	0	102	0	85	0	135	8	68	0	84
Winter Turf oats	0	83	0	18	0	87	0	95	0	91	0	82
Kanota oats	0	91	0	24	0	73	0	91	0	79	0	102
Agropyron spicatum	0	64	0	41								
Poa sandbergii	1	43	0	31								
Bromus rigidus	0	118	0	96								

TABLE 2.—*Species of Hendersonia and Wojnowicia on Gramineae, which have spore measurements approaching those of Wojnowicia graminis (McAlp.) Sacc. and D. Sacc.*

Fungus	Host	Year described	Spore size	Spore septation
			μ	
Hendersonia herpotrichia Sacc. (Pycnidial stage of *Ophiobolus herpotrichus*)	Gramineae	1883	36 × 6	8
H. hirta Schroet.	*Setaria verticillata*	1890	24–28 × 3–4	7
Wojnowicia hirta (Schr.) Sacc.	.....	.....	.....	.....
W. tenella Pat.	Gramineae	1897	25–26 × 4	7
H. graminis McAlp.	Wheat	1904	32–38 × 4–5	7
W. graminis (McAlp.) Sacc. and D. Sacc.	.....	.....	.....	.....
H. secalina Died.	Rye	1914	25 × 5	7
Angiopomopsis lophostoma Hoehn. *W. lophostoma* (Hoehn.) Sacc.	Phragmites	1912	.....	.....

The mean spore size of *Wojnowicia graminis* varies somewhat. McKinney illustrated, by photomicrograph, spores measuring 24–36 × 2.4–3.6 μ. The measurements made by the writer (50 spores samples) are of slightly broader spores. One field-grown, naturally inoculated sample (Wasco County, Oregon) averaged 29–36 × 4.6–5.7 μ; another, from artificially inoculated field material averaged 37.9 × 4.9 mean spore size (31–46 × 4.2–7.2 μ) and spores on an oat-barley-grain culture measured 26.4–39.6 × 3.2–5.4 μ. Van de Laar (11) reported pycnospores 29.58 × 3.47 μ and Broadfoot (2) gave 26.3–43.8 × 2.5–5.6 μ with a mean spore size of 36 × 3.9 μ. In general, specimens from Europe have somewhat smaller spores than those from the Pacific Northwest or from Australia. Those from Europe approach in size those of *Wojnowicia hirta* (Schr.) Sacc. Considering spore measurements alone, however, there is doubtful specific difference between any of the species listed in table 2.

Besides morphological differences, *Wojnowicia graminis* is a complex of physiologic forms that vary slightly, one from another, in rate and type of growth on potato-dextrose agar and on wheat-grain cultures and also in pigmentation and crystalline precipitates in potato-dextrose agar. The majority of the cultures associated with *Cercosporella herpotrichoides* were nearly identical in appearance, except for variations in substratum color (bright lemon, orange or rosaceous) but the isolates from the grasses and

those from wheat from the semiarid parts of the Columbia Basin free from *C. herpotrichoides* showed at least four more or less distinct strains. The strains from Australia were similar to strains isolated from wheat in Oregon. One of Dr. Ludbrook's cultures, namely, No. 8/248, he reports (in correspondence) is exceptional in that it fruits readily in pure culture. The usual paucity of fruiting agrees with the writer's findings in Oregon. In 1929–30, the writer made more than 150 isolations from wheat and grasses collected in Oregon and Washington. These cultures, on potato-dextrose agar, were kept for months at 15° C. and only one culture was found to be sporulating, which indicates the meagerness of sporulation under these conditions. A few cultures on potato-dextrose agar, however, have sporulated regularly (Fig. 2) when kept at 3.3° C. in an electric re-

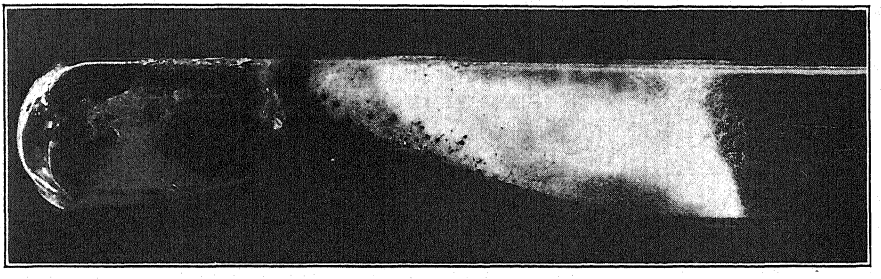


FIG. 2. Potato-dextrose agar culture of *Wojnowicia graminis*, isolated from wheat in Oregon, showing pycnidia. $\times 1\frac{1}{3}$.

frigerator. Furthermore, the fungus has been found to sporulate readily on sterile wheat heads, wheat kernels, or a mixture of barley and oats kernels when kept out of doors. In such cultures the fungus produces spores both in pycnidia and in acervuli. Corn meal also has proved to be a good medium for inducing sporulation.

DISCUSSION

Within recent years several of the fungi long recognized as severe foot-rot parasites of winter cereals have been shown to be less active than formerly thought, and other fungi, either unknown or obscurely known, have replaced them in part. *Wojnowicia graminis* is clearly one that has been overrated and should be listed among the weaker parasites. There is still need for exact study of this and other fungi in the rôle of weaker assisting companions to other much more active parasites. Inoculation studies with mixtures of pure cultures of *W. graminis* and *Cercosporella herpotrichoides* are in progress in Oregon. Preliminary observations indicate that *W. graminis* does not appreciably increase the damage done by *C. herpotrichoides*.

The complex studied by Foëx and Rosella (4, 5, 6) and coworkers, by Fellows and the writer (18, 19, 20, 21, 22), and by van de Laar (11) indicate the relative pathogenicity of the several fungi, which are sometimes associated, as follows:

Active parasites:	*Ophiobolus graminis* and *Cercosporella herpotrichoides*
Weak parasites:	*Ophiobolus herpotrichus* and *Leptosphaeria herpotrichoides*
Very weak, secondary parasite:	*Wojnowicia graminis*

SUMMARY

Wojnowicia graminis (McAlp.) Sacc. and D. Sacc, is a species with a number of physiologic forms or strains, with some varieties in morphological characteristics, and is very weakly pathogenic on a number of cereals and grasses.

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ASCOCHYTA BOLTSHAUSERI ON BEANS IN OREGON

RODERICK SPRAGUE

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INTRODUCTION

Ascochyta boltshauseri Sacc. was found on snap beans (*Phaseolus vulgaris*) near Astoria, Oregon, in September 1932, apparently its first reported occurrence in the United States. Through the courtesy of Dr. F. P. McWhorter of the Oregon Agricultural Experiment Station the material was given to the writer for studies supplemental to earlier reported work on leguminous Ascochyta species (7, 8, 11, 12).[1]

PURE CULTURE STUDIES

The initial growth on potato-dextrose agar was grey and felt-like, which gradually changed to a mottling of greys and fluffier buff areas. The mature colonies somewhat resembled those of *Ascochyta pisi* Lib. from *Vicia villosa* (12).

HOST RANGE STUDIES

Pot-grown, 3-weeks-old seedlings were inoculated with spore suspensions from pure cultures and incubated in spray chambers, as previously described (12). The data in table 1 is from an unpublished portion of the manuscript of experimental studies made at the University of Cincinnati between 1926 and 1929 (11) with O. T. Wilson as faculty adviser. In this work neither *Mycosphaerella pinodes* (Berk. and Blox.) Stone, nor any of the physiologic forms of *Ascochyta pisi* were able to attack *Phaseolus vulgaris*. *P. aconitifolius* and *P. aureus* were slightly susceptible to *M. pinodes* and to *A. pisi* isolated from pea (*Pisum sativum*) and *A. pinodella* (from pea).

Table 2 summarizes inoculation studies conducted at Corvallis, Oregon, with the Ascochyta from beans collected at Astoria. The plants in this instance were 8 weeks old. *Phaseolus* spp. only were susceptible to the Ascochyta from beans, although there was some slight etching on the leaves of peas. Reisolations, however, were negative in the case of the pea.

Symptoms began to appear on the inoculated beans at Corvallis at the end of 5 days, well-formed lesions were present 2 days later, and pycnidia with maturing spores 3 days after that.

[1] Credit for first distinguishing *Ascochyta pisi* Lib., *A. pinodes* L. K. Jones and *A. pinodella* L. K. Jones from each other belongs to M. B. Linford and F. R. Jones who were aware of the difference for some time before any published data, the first of which was published by Linford in The Canning World (7). L. K. Jones later confirmed this work and rounded out the studies.

TABLE 1.—*The results of inoculating Phaseolus spp. with various Ascochyta spp. and Mycosphaerella pinodes at Cincinnati, Ohio (1926–1929). Plants were inoculated with pycnospore suspensions and incubated in a spray chamber. All noninoculated check plants were healthy. All plants were grown in six-inch clay pots*

Technical name of host	Common name of host	Results, Positive (+) and Negative (−) by number of pots									
		A. pisi (pea)[a]		*M. pinodes* (pea)		*A. pinodella* (pea)		*A. pisi* (Vicia faba)		*A. pisi* (V. villosa)	
		+	−	+	−	+	−	+	−	+	−
Phaesolus aconitifolius	Moth bean	1	2	2	0	2	1	0	1	0	0
Phaseolus angularis	Adzuki bean	0	3	0	3	0	2	0	3	0	0
P. aureus	Mung bean	3	1	2	0	3	1	0	1	0	0
P. calcaratus	Rice bean	0	2	0	3	0	4	0	1	0	0
P. lunatus	Sieva bean	0	4	0	2	0	3	0	3	0	0
P. limensis	Lima bean	0	3	0	1	0	1	0	2	0	0
P. mungo	Urd bean	0	4	0	3	1	1	0	1	0	0
P. coccineus	Scarlet runner bean	0	4	0	2	0	2	0	2	0	0
P. vulgaris	Black valentine snap bean	0	3	0	2	0	3	0	3	0	0
P. vulgaris	Red Mexican field bean	0	3	0	3	0	0	0	2	0	3
P. vulgaris	Lazy wife snap bean	0	3	0	3	0	1	0	1	0	1
P. vulgaris	Refugee snap bean	0	3	0	2	0	1	0	0	0	0
Total		4	35	4	24	6	20	0	20	0	4

[a] The name in parenthesis refers to the host from which the inoculum was originally isolated. *A. pisi* from pea was isolated from a diseased pod from Ohio, *M. pinodes* from Wisconsin seed, *A. pinodella* from Wisconsin, *A. pisi* from nine year old *V. faba* seed from California and *A. pisi* from *V. villosa* leaf spot, Pullman, Wash.

MORPHOLOGY

The morphological characters of the material of *Ascochyta boltshauseri* from Astoria is as follows: pycnidia numerous, obscure, light brown to dark brown (on pods) 120 to 150 μ in diameter; pycnidia on inoculated leaves golden brown, 60 to 130 μ in diameter. Spores, on pods, predominately one-septate (many immature), 15 per cent to 20 per cent of the spores were 2- to 5-septate, mostly 2-septate; one septate spores 10–27 × 2.5–6.6 μ, mean spore size 18 × 4.5 μ; multiseptate spores 16.6–34 × 4.5–7.1 μ, mean spore size 21.5 × 5.6 μ; spores in pure culture on potato-dextrose agar mostly nonseptate, constricted in 2 or more places, 6–11 × 2–3 μ, a few much larger; spores on lesions from artificially inoculated leaves the same size as on pods of naturally inoculated material.

TAXONOMY

While certain forms of *Ascochyta pisi* approach in spore size those of *A. boltshauseri* the two are separate species as they have entirely distinct

TABLE 2.—*The results of inoculating various legumes at Corvallis, Oregon, with Ascochyta boltshauseri on May 1, 1933, and incubating in a spray chamber until May 11, 1933. All noninoculated check plants were healthy. Water temperature 10–14° C.*

Technical name of host	Common name of host	Results, Positive (+) or Negative (−)						
		Number of pots of plants		Number of plants		Number of leaves		Relative severity
		+	−	+	−	+	−	
Phaseolus vulgaris	Red valentine bean	2	0	2	0	16	0	Moderate
	Boston navy bean	2	0	8	0	36	2	Slight+
	Bush black valentine bean	2	0	9	0	42	0	Severe+
P. aureus	Mung bean	2	0	6	0	25	0	Dying
P. angularis	Adzuki bean	2	0	9	0	42	0	Moderate
P. coccineus	Scarlet runner bean	2	0	9	0	51	4	Severe+
Pisum sativum var.	Alaska pea	2	2	4	7	6	105	Trace (?)
	Blue Prussian field pea	0	2	0	9	0	136	
Vicia villosa	Hairy vetch	0	2	0	21	0	460	
Vicia sativa	Common vetch	0	1	0	19	0	560	
Medicago sativa	Grimm alfalfa	0	2	0	39	0	218	
Trifolium repens	White clover	0	1	0	19	0	540	
Lathyrus odoratus	Sweet pea	0	2	0	10	0	112	

host ranges and the dark to drab zonate lesions of *A. boltshauseri* are entirely distinct from the light-colored red-bordered spots of *A. pisi*. The wide difference in spore size and distinct host range alone are sufficient to separate *A. boltshauseri* and *M. pinodes*.

Stagonopsis phaseoli Eriks. is sometimes reported as a parasite of beans in Europe. The type, as examined by the writer (Fungi par. scan. 377), has spores averaging 19.5×5.9 μ and the symptoms are identical with those of *A. boltshauseri*. The two fungi are unquestionably identical. *Stagonopsis phaseoli* was described in a publication appearing Sept. 9, 1891 (5). The description of *A. boltshauseri* appeared in Heft 3 of the Zeitschrift für Pflanzenkrankheiten of 1891 (1). While Boltshauser-Amrisweil dated his article June 27, 1891, there is no apparently possible source of information on when this article actually appeared. The present editor of this journal, Dr. Tubeuf, says in answer to a letter of inquiry by the writer, *Heft 3 ist nominell zwischen 1 Mai u. 1 Juli, in Wirklickheit nahrscheinlich etwas verspätet, aber doch in Laufe des Juli 1891 erschienen.* While there may be some question as to whether the article, signed June 27, appeared as early as July it is reasonable to assume that it appeared some time before Eriksson's did. Inasmuch as Saccardo's name is generally accepted the writer prefers to retain it until definite proof is advanced that Eriksson's article appeared first.

The work of Diedicke (3, p. 142, 4 pp. 388–389, 397–400) in proposing *Stagonosporopsis boltshauseri* (Sacc.) Died. and of Petrak in making the combination *Stagonosporopsis hortensis* (Sacc. and Malbr.) Petrak (9) is not acceptable to the writer, as he considers Stagonosporopsis Died. as invalid (12). Since the most common condition of mature material of this fungus is that of an Ascochyta, the writer considers that it should be assigned to that genus.

The type material of *Stagonospora hortensis* Sacc. and Malbr. is not available. The original description (10) states that the spores are 4-guttulate, finally 3-septate, hyaline, and measure $18–27 \times 7\ \mu$. The writer suspects that this fungus might be the same as *A. boltshauseri* but on account of not having type material of *S. hortensis* he hesitates to follow Petrak's lead. There is no indication in Petrak's work (9) that he saw the type of *Stagonospora hortensis.*

Stagonospora phaseoli Dearness (2) is distinct from *Ascochyta boltshauseri.* Type material furnished by Prof. Dearness had light-colored, red-bordered lesions and typical multiseptate Stagonospora spores.

The synonomy of *A. boltshauseri* is as follows:

Ascochyta boltshauseri Sacc. Midsummer 1891

Syn.

Ascospora pisi Fuckel 1869 (6)

Stagonopsis phaseoli Eriks. Sept. 9, 1891

Stagonosporopsis boltshauseri (Sacc.) Died. 1912

Stagonosporopsis hortensis (Sacc. and Malbr.) Petrak 1921

SUMMARY

1. A collection of *Ascochyta boltshauseri* Sacc. on snap beans (*Phaseolus vulgaris*) was made near Astoria, Oregon, in 1932, apparently the first report of this disease in North America.

2. Cross inoculation studies showed that *A. boltshauseri* was distinct from other Ascochytae on legumes.

3. *Ascochyta boltshauseri* and *Stagonopsis phaseoli* Eriks. which have been considered two distinct diseases are synonymous. Since Eriksson's description apparently appeared a few weeks later than that of *A. boltshauseri* the latter name is retained.

4. The need for comparative study with *Stagonospora hortensis* type material is pointed out. The invalidity of the genus Stagonosporopsis Died. is again mentioned.

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RELATION OF SOIL ACIDITY TO A SEEDLING DISEASE OF ALFALFA ON THREE IOWA SOILS[1]

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(Accepted for publication October 26, 1934)

In 1933 a high correlation between soil acidity and a damping-off disease (Pythium species) of sugar beets was noted in certain fields in northern Iowa. At the same time similar acid soils were observed to yield poor stands of alfalfa. These observations brought to mind the diverse explanations for failures of alfalfa seedings on acid soils and suggested the possible importance of damping-off fungi. In order to determine this rôle of damping-off fungi, trials were started with alfalfa on acid (pH 6.2) and neutral (pH 6.8 to 7) samples of Webster silt loam under greenhouse conditions. The alfalfa seedlings in acid soil showed symptoms similar to those induced on sugar-beet seedlings by species of Pythium. Some embryos were invaded and reduced to a gelatinous consistency before they had broken the seed coat. Others were killed before they emerged above the soil line. Easily visible mycelium extended from these dead plants to the surrounding soil particles. When the few plants that did emerge made their appearance above the surface of the soil, they were already infected. The first noticeable symptom on these plants was a collapse of the primary root and the lower portion of the hypocotyl. They quickly became flaccid, wilted, and died.

On neutral soil in the greenhouse the general effect was much less severe. Several pots of seedlings were grown apparently free from invasion by Pythium. Infection, when it occurred, very seldom if ever took place before the seedlings emerged from the soil, and even then a large percentage escaped.

In the field the effect was not quite so severe on either acid or neutral soil. On acid Webster silt loam, a few of the seedlings were killed before emergence. Some were attacked just as they emerged. Here again the first noticeable symptom was the collapse of the infected tissues of the hypocotyl and primary root. In young seedlings, invasion apparently was very rapid and general so that the upper hypocotyls were cut off from a moisture supply and under dry conditions the plants wilted and died in less than 24 hours.

On seedlings infected when older, parasitic invasion seemed restricted and the amount and rate of necrosis less extensive. The hypocotyl and primary root and sometimes the secondary roots were partially or com-

[1] Journal paper No. J189 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 77.

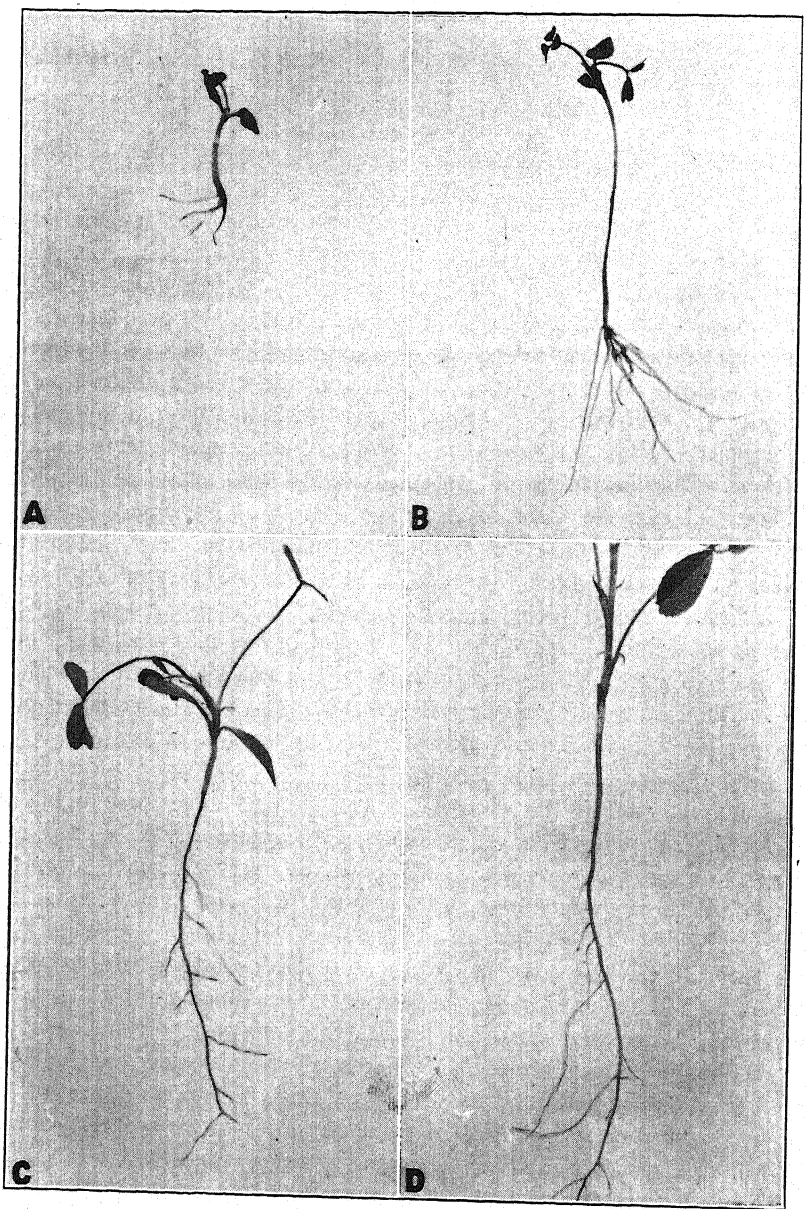


FIG. 1. Alfalfa seedlings 3 weeks old grown on acid Webster silt loam, showing different types and degrees of injury by damping-off parasites. A. Seedling with primary root and lower hypocotyl rotted off when small. B. Larger seedling with primary root and 3 branch roots rotted off; in each case one or more branch roots have arisen from living tissues above the lesion: there is also a cortical lesion on the hypocotyl. C. Cortical lesion on the primary root and lower hypocotyl of a larger seedling; infection apparently occurred later than in A. D. A healthy seedling. ×1.

pletely girdled (Fig. 1; A, B, C). Frequently the tip of a primary root had been destroyed and several branch roots had grown out from the healthy tissue above (Fig. 1; A, B). Primary roots of diseased seedlings produced fewer secondary roots than did those of healthy seedlings. These older seedlings persisted, even though they were less vigorous than healthy ones. The first symptoms quickly disappeared as the collapsed cells of superficial lesions turned brown and disintegrated. As a result of such exposure, the remaining central portions of the axes appeared rough and desiccated.

All these symptoms occurred on plants grown on neutral Webster silt loam in the field, but to a lesser degree and in a smaller number of cases. On this neutral soil 6.4 per cent of the small seedlings died, as compared to 16 per cent on acid soil in the same plot. Since these plantings were not dug, the above figures do not include the plants that might have had lesions but were not killed immediately. On Clarion loam, where seedlings that were killed in early stages were not counted, only 59 per cent of the plants remaining on acid soil were healthy 5 days after emergence, whereas, on neutral soil in the same field under the same conditions, 93 per cent of the remaining plants were healthy. On acid Tama silt loam, about 2 weeks after emergence, only 52 per cent of the remaining plants could be classified as healthy and some of these were doubtful. To date it has not been possible to follow alfalfa seedings through to the first cutting and record continuous differences on acid and neutral soils, but comparative stand counts have been made on soils of different degrees of acidity. These counts show that acid soils have thinner stands than do neutral soils. A field seeding of alfalfa at Kanawha, Iowa, had an average of 7.1 plants per square foot on soil with a lime requirement of one and one-half tons per acre as compared to 12.3 per square foot on neutral soil. A field in Jones county on acid Clinton silt loam had one of the better stands in the neighborhood with only one plant per square foot. In 2 fields of alfalfa on one farm in Mitchell county stands were satisfactory only on naturally neutral soil, even when some lime had been applied to both neutral and acid areas.

The cultures isolated from recently collapsed tissues of infected seedlings appear to be species of the genus Pythium. Their young mycelium branches freely, is nonseptate, relatively hyaline, and from 1.5 to 3.5 μ in diameter. Older mycelium is larger and septate and devoid of protoplasm. Spherical hyaline conidia or sporangia, 18 to 34 μ in diameter, are borne terminally on branches of the mycelium. Intercalary ellipsoid conidia are about the same size. Zoospore formation has not been observed and oöspores have not yet made their appearance on artificial culture media. Recently infected material yields a high percentage of cultures on potato-

dextrose agar with ordinary seedling isolation technique. A delay of 24 hours after the appearance of initial symptoms often results in a poor yield. Very young infected seedlings sometimes dry up in 3 to 6 hours. Whether or not all the cultures obtained are of one species has not been determined, but it is felt that they are a rather homogeneous group. It is also believed that the same parasite or parasites that attack alfalfa will attack beets and other crops in the same soil. Beets that were planted alongside of alfalfa on Webster silt loam, Clarion loam, and Tama silt loam exhibited disease symptoms similar to those on the alfalfa, and isolation trials from diseased beet seedlings yielded cultures of Pythium similar in growth habit and conidia production to those obtained from alfalfa. When planted in the greenhouse on acid Webster silt loam on which sugar beets are severely diseased in the field, alfalfa, yellow sweet clover, red clover, alsike clover, white Dutch clover, *Lespedeza* sp., *Dalea* sp., and sugar beets all damped off and yielded cultures of similar pythiaceous fungi upon isolation. In the field on this soil the disease has been noted on red clover, sweet clover, alsike, and flax in addition to alfalfa and sugar beets, and successful isolations of similar fungi have been recorded from field specimens of all these except sweet clover.

Acid Webster silt loam, when steamed for 2 hours at 15 pounds, will grow a higher percentage of healthy alfalfa seedlings than will neutral soil not steamed. An application of 50 cc. of 0.5 per cent formaldehyde in a 4-inch pot will produce results similar to those noted on steamed soil. Limestone applied abundantly to this acid soil in the greenhouse has inhibited damping-off of alfalfa. Hydrated lime dusted on the seed has repeatedly given the same results with sugar beets.

In the accompanying table is a sample of the kind of results obtained by placing pots of alfalfa and alsike-clover seedlings at two temperatures, one below, the other near the optimum for the growth of Pythium in pure

TABLE 1.—*Results obtained from pot cultures of alfalfa and alsike-clover seedlings at two temperatures, one below, the other above the optimum for Pythium in pure culture*

Soil: Webster silt loam	Alfalfa				Alsike			
	20–25° C.		9° C.		20–25° C.		9° C.	
	H[a]	D[b]	H	D	H	D	H	D
Acid soil (pH 6.2)	1	28	28	30	93	30	132	23
Acid soil steamed (pH 6.4)	152	0	159	0	139	0	141	0
Neutral soil (pH 6.8–7.0)	96	34	106	8	148	0	134	0

[a] H = healthy. [b] D = diseased.

culture. Both these legumes emerged in larger numbers at the lower temperature and a higher percentage of the emerged plants were healthy. Steamed acid soil yielded a healthy stand of what was probably the maximum number of seedlings that would grow from the 200 seeds planted. While not entirely free from infection, the number of plants obtained on neutral soil approached the maximum and there was only a small increase in number of healthy plants on neutral soil at the lower temperature. In the greenhouse, alsike repeatedly has given a higher percentage of healthy plants on acid Webster silt loam than has alfalfa. Alsike is considered a better acid-soil legume than alfalfa. The symptoms on alsike and isolations from infected alsike plants were similar to those of alfalfa.

SUMMARY

It has been established that what appears to be Pythium species attacked germinating, emerging, and young growing seedlings of alfalfa on 3 acid Iowa soils in greater numbers and with greater damage to individual plants than on 2 similar neutral soils. The same disease has been found in the field on sugar beets, other legumes, and flax. It has also been determined that at 9° C. young alfalfa and alsike seedlings damped off less than at 20–25° C. Steaming and formaldehyde treatment enabled alfalfa and alsike seedlings to establish stands of healthy plants on acid soil as large or larger than those established on undisturbed neutral soil. The same effect to a limited degree was brought about by hydrated lime dusted on sugar beet seed and in one alfalfa planting by limestone, generously applied to the soil.

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CYTOLOGY OF SECONDARY SPORE FORMATION IN GANODERMA

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(Accepted for publication September 29, 1934)

In 1919 White (9) recorded that there was not a single instance of conidia or secondary spore formation from any part of the sporophore of *Ganoderma applanatus* (Pers.) Wallr. But I have observed that usually at the end of the rainy season (in October and November) the basidia of *G. lucidus* (Leyss.) Fr. and *G. applanatus* (Pers.) Wallr. are gradually succeeded by tramal-hyphae elongations within the pore tubes, which ultimately bear at their summit asymmetrically secondary spores that are indistinguishable from ordinary basidiospores in shape, size, and ornamentation of the spore walls. Buller (3, p. 202) would rightly call them secondary basidiospores, as, like basidiospores, they are asymmetrically situated on the end of the stalk and are shot away by the drop-excretion mechanism. To start with, after the rains a number of white hyphal projections bearing 2 conjugate nuclei with distinct clamps are found here and there amongst the basidia, some of them are almost uniformly narrow (Fig. 1, *a*), others have sickle-shape hyphae or straight broader hyphae at the base with narrow hyphae at the top (Fig. 1, *b–d*), bearing a number of clamps; the sickles are almost as long as basidia but are much narrower (about one-fourth the breadth of the basidia), they might be regarded as intermediate between basidia and tramal hyphae. These tramal-hyphae projections are then surmounted by very small rudimentary spores that gradually enlarge and assume the form and color of mature basidiospores. Finally, the basidia are hardly seen, the pore tubes show a large number of tramal-hyphae projections bearing secondary spores at their summit. Such tramal-hyphae projections with secondary spores in large numbers I have seen also in cases of *Trametes latinea* Berk. and *Polyporus calcuttensis* Bose collected in November and December, 1931, and of *P. orchroleucus* Berk. during the abnormally dry period of August, 1934. From the first-named one, *i.e.*, *T. lactinea*, I could get copious spore discharge within an agar plate, though there was not a single basidium within any of the pore tubes. In some years, even during the rainy seasons when there were intervals of drought, I could get basidia and tramal-hyphae projections with secondary spores side by side within the pore tubes of these Ganoderma species, whereas conversely, when there were unexpected showers of continuous rain for about a week in November, 1931, I found that the tramal-hyphae projections had all disappeared and we had only basidia within their pore tubes. As years pass on, I believe many more will probably be added to

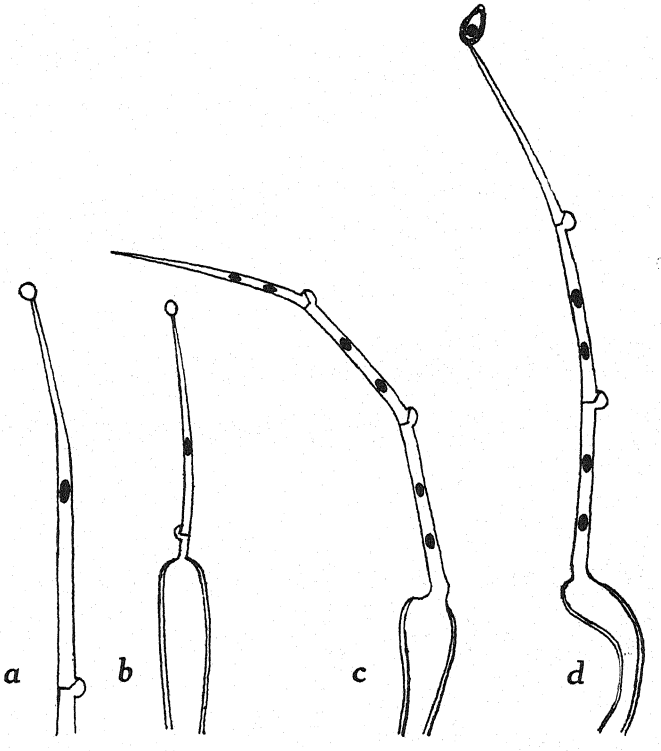


FIG. 1. Types of hyphal projections among basidia.

the list of Polypores that reproduce by such secondary spores after the rains or during the dry intervals of the rainy season.

Fixing hymenial portions of fresh sporophores of *Ganoderma lucidus* and *G. applanatus* with tramal-hyphae projections, bearing secondary spores after the rains, in Bouin's fluid and finally embedding them in paraffin and staining microtome sections (6–10 μ thick) with Heidenhain's iron haematoxylin and gentian-violet (Dorothy Newton's method) it was found that the tramal-hyphae projections are originally binucleate. Then the 2 nuclei in the hyphae gradually fuse into one fusion nucleus, which fragments usually into 4 or 5 pieces (mere black dots). The fragments then pass out of the elongated hyphae into the terminal spore, where the nucleus of the spore seems to be constructed from these chromatin fragments. All these stages have been figured in the accompanying camera-lucida sketches (Fig. 2). No mitotic stages could be traced. So the mode of formation of secondary spores seems to be by way of amitosis.

The amitotic formation of normal tissue is by no means without parallel. Guilliermond (6) has shown that, in the case of yeasts, budding seems to

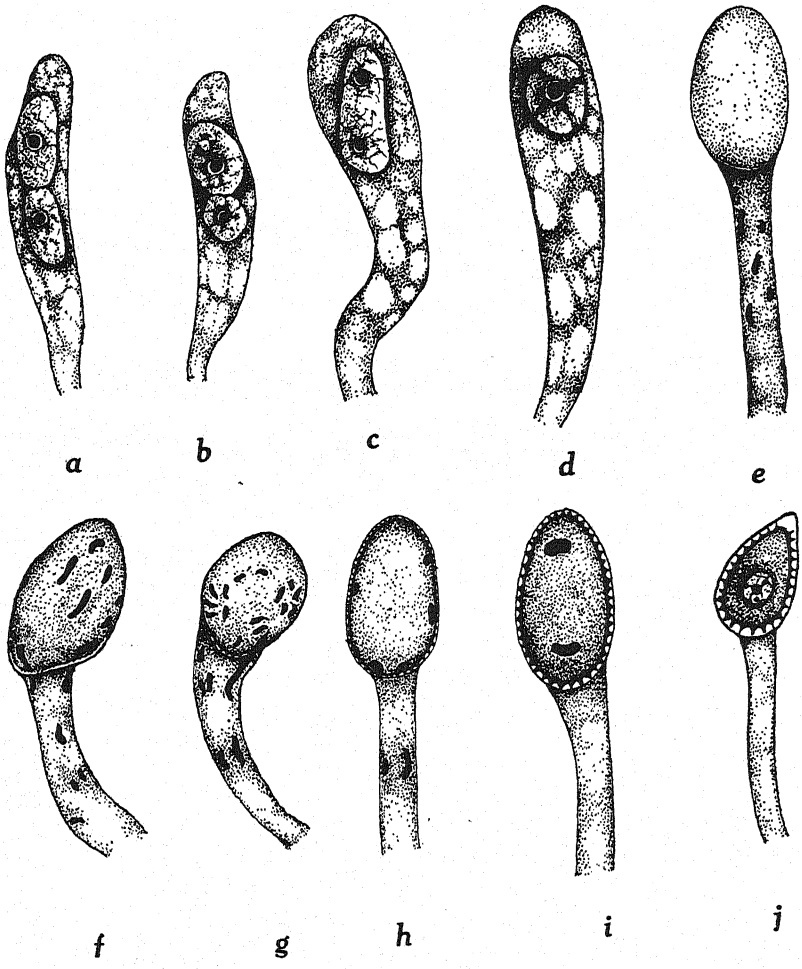


FIG. 2. *a–d.* Tramal hyphae with nuclei. *e–j.* Tramal hyphae with sectioned spores in different planes. Detailed explanation is given in the body of the text. × about 1800.

take place by amitosis. In the case of *Sporobolomyces roseus,* Buller (3, pp. 191–193) has recorded that at the time of spore formation and of the maturity of the spore the nucleus of the yeast cell passes up to the sterigma and there becomes constricted into 2 daughter nuclei evidently by way of amitosis. In the cytologic study of Synchytrium, Griggs (5, pp. 127–138) holds that the division of the primary nucleus is by the process of amitosis. The formation of reproductive cells, independent of mitosis in certain specific cases, must affect the current theories of heredity, which, according to Mendel's law, lean very heavily on the individuality of the chromosomes and their separation in the reduction division. In this connection Griggs

(5) remarks: " . . . It is obvious that our theories of heredity will require considerable revision."

Brefeld (2) regarded the simple conidiophore with few conidia in *Fomes annosus* as the ancestral form of basidium; but, unfortunately, conidiophores had not been investigated cytologically, nor had the mode of formation of conidia from the tips of the conidiophores. Hence, any comparison is not possible. On the other hand, Buller (3) remarks that conidia are all unlike basiodospores, as they are probably not shot away by the drop-excretion method. Hiley (7, p. 105) also is of the opinion that, cytologically, there probably is great distinction between the modes of formation of conidia and basidiospores.

Chamberlain (4) has recently remarked that free nuclear division is a phenomenon arising from the fact that the nucleus present was too small to segment the comparatively large mass of cytoplasm. In connection with growth of protoplasm, Sharp (8) also remarks that a certain proportion of nuclear and cytoplasmic substances (the "nucleo-plasmic ratio") must be maintained. Bergman (1) holds almost the same view that "the nucleo-plasmic coefficient varies with the absolute size of the nucleus."

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PHYTOPATHOLOGICAL NOTES

A Probable Case of Sulphur Starvation in Tobacco.[1]—On August 31, 1934, while examining tobacco in Owen County, Kentucky, a peculiar chlorosis of the tips of the upper first to third leaves of topped White Burley tobacco plants was noticed in several fields, and a few days later the same condition was observed in fields in Nicholas County. The affected leaves were green at the base, but the tips were grayish yellow, the yellowing sometimes extending well down on the edges of the leaves (Fig. 1, A). The areas in which affected plants were found were hilly and subject to considerable erosion. The soil was hard and cracked. Chlorosis was most severe on the slopes, and least on the ridges, but did not occur on newly cleared land or land to which a mixed fertilizer had been applied for the tobacco crop. The growers were familiar with the trouble and claimed that it occurred in years when tobacco made better growth than would be expected from the fertility of the soil and where commercial fertilizers had not been used.

It was evidently a deficiency trouble, and the symptoms very closely resembled those attributed to sulphur deficiency, but were unlike any of the other deficiency troubles we have been able to produce in tobacco in sand and water cultures in the greenhouse. The rainfall record for the summer favored sulphur starvation as the most probable cause of chlorosis. The season was dry following setting and, until about 3 weeks before the observations were made, when there was a very heavy rain in a comparatively short time with a great deal of run-off. The tobacco had made most of its growth since that time. Thus, although there is ordinarily sufficient sulphur carried down with the rain in Kentucky[2] to satisfy crop requirements, the amount carried down by a single heavy rain would be much less than by several lighter rains occurring over a longer period of time. Karraker and Bortner[3] demonstrated that sulphur starvation sometimes develops in tobacco grown in pots in soil from the Central Bluegrass area of Kentucky, if only a small volume of soil be used to which no sulphur-containing fertilizer has been added. Analyses of a large number of soils, made by Shedd,[4] show a much lower percentage of sulphur in subsoils than in sur-

[1] Published by permission of the Director of the Kentucky Agricultural Experiment Station.

[2] Johnson, E. M. Sulfur in rainfall in Kentucky. Jour. Amer. Soc. Agron. 16: 353–358. 1924.

[3] Karraker, P. E., and C. E. Bortner. Studies of frenching of tobacco. Kentucky Agr. Expt. Sta. Bull. 349, 1934, see page 99.

[4] Shedd, O. M. The sulphur content of some typical Kentucky soils. Kentucky Agr. Expt. Sta. Bul. 174. 1913.

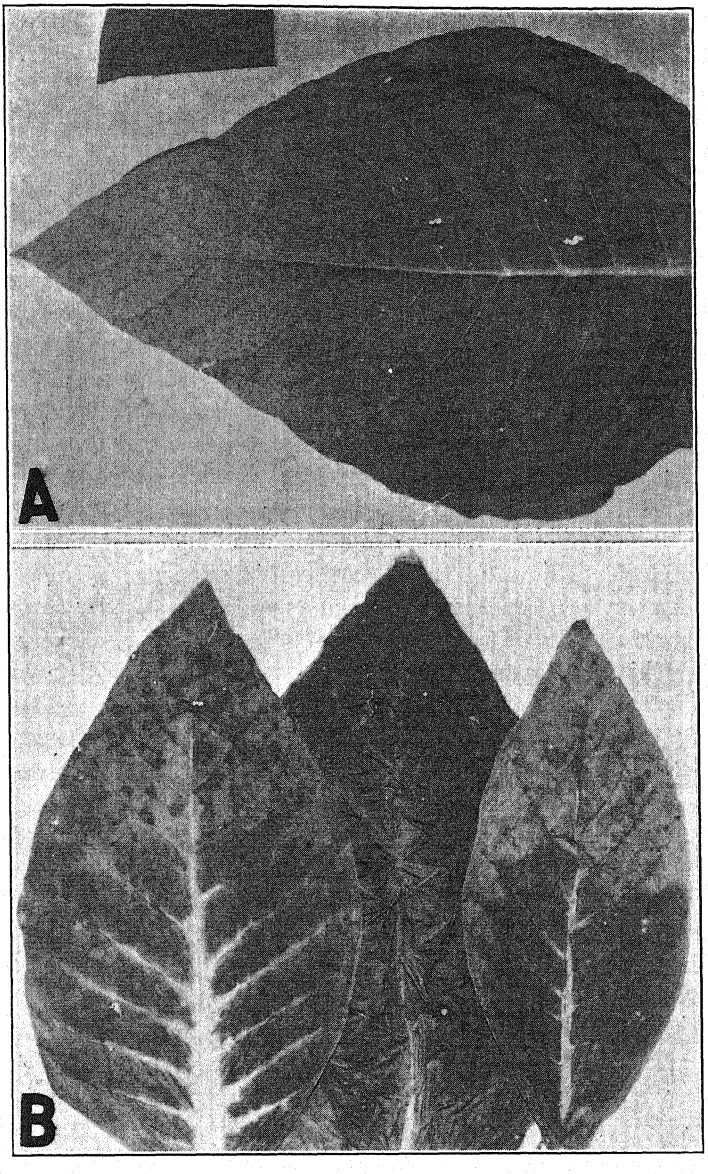


FIG. 1. A. Uncured leaf of white Burley tobacco collected in Owen County, Ky. The chlorotic tip closely resembles sulphur deficiency. A small bit of leaf from a normal green leaf is shown for comparison. B. Two chlorotic leaves from Owen County and a normal leaf (center) from the station farm when nearly cured. The mid and lateral veins of the chlorotic leaves were still partly alive. The areas that were chlorotic before curing show clearly in the cured leaf.

face soils of cultivated areas. The erosion already noted had removed most, if not all, of the original surface soil, as indicated by the clayey nature of the soil where the deficient leaves were found.

Affected leaves from Owen County and normal leaves from the Station farm were brought to the laboratory for observation while curing and for chemical analysis. Some of the affected leaves were cured whole. The chlorotic tips were removed from others, and both the tip and butt portions cured. The chlorotic tips yellowed and died very quickly. Affected whole leaves, however, yellowed very slowly in comparison with normal leaves and dried out much more slowly. In 12 days the normal leaves were completely browned and had been for several days, while the affected leaves and the severed butts of affected leaves were just beginning to brown. After 20 days the midveins of 7 of 10 of the normal leaves were dry, whereas the midveins of 10 affected leaves were green and turgid nearly the length of the leaves. There was also a strip of blade tissue still alive along the basal third of the midveins of some of these leaves (Fig. 1, B).

When cured, the normal leaves became a uniform cinnamon to Kaiser brown, while the chlorotic areas of affected leaves turned chamois to honey yellow (Ridgeway), grading off to cinnamon brown in the more normal-appearing areas. The grower on whose farm the specimens were collected stated that his tip leaves always sold well.

Chemical analyses of the affected tips, butts of the same leaves, and the normal leaves were made by O. M. Shedd, of the Department of Chemistry of the Experiment Station. Nitrogen, nicotine, crude ash, phosphorus, magnesium, potassium, and sulphur (0.42, 0.56, 0.61 per cent sulphur) each increased in the order of chlorotic tips, butts of affected leaves, and normal leaves, the latter being highest. Iron was the same in affected tips and normal leaves but slightly lower in butts of affected leaves. Manganese was highest in the normal leaves (0.0089), next in the affected tips (0.0037), and least in the butts of affected leaves (0.0026 per cent). Calcium was lowest in affected tips. Chlorine was highest (0.52 and 0.49 dup.) in the butts, least in normal leaves (0.10), and intermediate in the affected tips (0.17 per cent). The high chlorine content of the butts of affected leaves may account for their slow curing.—W. D. VALLEAU, Kentucky Agricultural Experiment Station, Lexington.

Nonsterile Soil Leachate Stimulating to Zoosporangia Production by Phytophthora sp.[1]—A copious production of papillate zoosporangia of *Phytophthora cinnamomi* Rands has been obtained consistently from myce-

[1] Published with the approval of the director as Miscellaneous Paper 14 of the Pineapple Experiment Station, University of Hawaii.

lial mats grown for 4 days in malt broth[2] at 25° C.—27° C., subsequently washed in sterile distilled water, and incubated at 21° C.—25° C. in nonsterile soil leachate.

Zoosporangia of this species described by other authors have been in all cases of the nonpapillate type. They are produced only scantily and with difficulty by the methods of Petri,[3] Leonian,[4] and Waterhouse.[5] This difficulty is largely responsible for the erection of the new genus Pseudopythium by Sideris, as a designation for his isolations of *P. cinnamomi.*[6]

Mycelium grown in malt broth is better suited for the production of zoosporangia than that grown in Leonian's succinic acid medium, Waksman's broth, green pineapple fruit decoction, cubes of green pineapple, or on any of the following agars: papaya, malt, corn meal, oats, Ranker's, or Czapek's.

The differences in abundance of sporangia in sterile distilled water, tap water, M/100 KNO_3, dilute malt broth, or sterile soil leachate and that in nonsterile soil leachate have been repeatedly of the order illustrated as concentrations II or III and V respectively by Leonian.[4] The results of experiment CF–31 summarized in table 1 are characteristic of these differences.

The factors in nonsterile soil leachate responsible for stimulation of the production of zoosporangia have not yet been learned, but the results of experiments conducted for that purpose are suggestive.

It was first learned that the failure to add iron to sterilized soil leachate or M/100 KNO_3 was not a limiting factor. The age of mycelium ranging from 72 to 120 hours exerted no measurable influence. Essentially similar quantities of sporangia were formed at 21° C., 25° C., and 29° C. (but none at 35° C.) in the leachates of 3 soils. They were found to occur abundantly only in the nonsterile extracts of these soils, having pH values of 5.9, 6.8, and 7.2 respectively, being most abundant at pH 6.8. Scarcely any were formed in the sterile leachates having pH values of 6.4, 7.3, and 7.6. Copious production of sporangia occurred in all leachates from soils dried at 43° C. for 24 hours and at 27.5° C. for 72 hours. Both combinations of

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TABLE 1.—*Factors favoring zoosporangial production in strain 60 of Ph. cinnamomi (Sideris' Pseudopythium). Concentrations of sporangia scored according to Leonian's figures (p. 450)*[a]

Nutrient medium[b]	Soil solution		Score for 3 plates
	Sterility	Quantity	
1	sterile	1.5 cc.	9
1	not sterile	1.5	12
1	sterile	3.0	3
1	not sterile	3.0	15
1	sterile	6.0	9
1	not sterile	6.0	15
2	sterile	1.5	6
2	not sterile	1.5	8
2	sterile	3.0	4
2	not sterile	3.0	3
3	sterile	1.5	2
3	not sterile	1.5	9
3	sterile	3.0	0
3	not sterile	3.0	5
3	sterile	6.0	0
3	not sterile	6.0	4

[a] See footnote 4.

[b] Mycelial mats were grown for 96 hours in nutrient medium (1) malt broth, or (2) green pineapple decoction, or (3) Leonian's succinic acid medium. Following washing in sterile distilled water, they were incubated in 100-mm. Petri dishes of soil solution of the quantity and sterility indicated.

time and temperature had previously been shown to kill the Phycomycetes occurring in the soil. No sporangia were found in steam-sterilized leachates of the same soil.

A more intensive study was then made of the effect of different modes of sterilizing the soil prior to leaching or of sterilizing the leachates of nonsterile soil. The method of Pistor[7] for producing bacteriological sterility of soil without markedly affecting solubility of the ash, or changing total acid or pH, MgO nitrogen, total nitrogen, or HCl (Sp. g. 1.19) hydrolizable nitrogen was compared with other methods shown in table 2 using a single soil.

Other species of Phytophthora causing pineapple heart rot also produce sporangia most abundantly in nonsterile soil solution.

From these studies it appears probable that organisms other than Phycomycetes present in nonsterile soil leachate may bring about conditions most favorable for the production of zoosporangia by *Phytophthora* sp.

[7] Pistor, R. Beiträge zur Kenntnis der biologischen Tätigkeit von Pilzen im Waldboden. Centralbl. Bakt. Abt. II, 80: 378–410. 1930.

TABLE 2.—*The comparative effects of various modes of sterilizing soil leachate on the production of zoosporangia*

Number	Leachate preparation[a] Type	pH	Abundance of zoosporangia
1	Steam sterilize soil, 250° F. for 2 hours, then leach	6.9	None
2	Sterile soil by Pistor's method, then leach	5.9	None
3	Leach unsterilized soil: sterilize leachate, 250° F., 20 minutes, do not aerate	6.7	None
4	Aerate aseptically an aliquot of sterile leachate from treatment 3	4.9	Scant
5	Aliquot of leachate from (3) unsterilized	5.9	Abundant
6	Sterile Petri's[b] mineral solution instead of soil solution	6.2	Scant
7	Inoculate 100 cc. of Petri's solution with 1 cc. aliquot of (5), incubate 24 hours	.....	Abundant

[a] Quadruplicate 100 mm. Petri dishes were aseptically prepared following the treatment in bulk of each leachate.

[b] $Ca(NO_3)_2 \cdot 4H_2O$—0.40 gm.; KH_2PO_4—0.15 gm.; $MgSO_4 \cdot 7H_2O$—0.15 gm.; KCl—0.06 gm.; distilled water—1 liter.

The presence or absence of papillae on the sporangia of *Phytophthora cinnamomi* appears to be a variable character dependent upon environment for expression. It is, therefore, of doubtful taxonomic value.—F. P. MEHRLICH, Pineapple Experiment Station, University of Hawaii, Honolulu, Hawaii.

Gloeosporium Leaf Spot, a Serious Disease of Orchids.—Plants of *Pholidota imbricata* Lindl. growing in the floricultural greenhouses of the University of Illinois were found to be severely infected with a disease of the foliage (Fig. 1, A, B). The disease may originate at many points on the leaf by the germination of scattered conidia of the causal organism, with resultant prominent, sunken, reddish brown spots with a definite margin. These spots eventually coalesce and in many cases destroy the entire leaf, which becomes brown and dry. In several instances it was observed that the disease may originate somewhere near the apex of the leaf and gradually spread backwards towards the pseudobulb, destroying the blade in its entire width as it travels.

Infection may take place on either side of the leaf, as evidenced by the minute specks characteristic of the early stages of the disease. The tissues

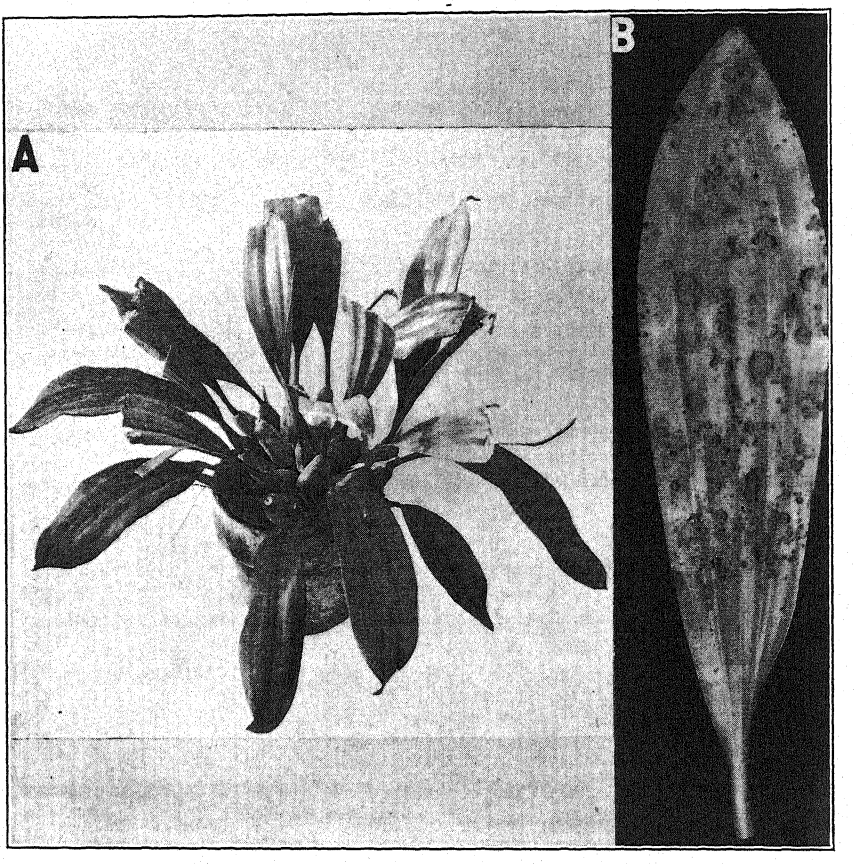


FIG. 1. A. Plant of *Pholidota imbricata*, showing severity of infection with Gloeosporium. Note that the tips of numerous leaves have been removed in an attempt to prevent the further spread of the disease. B. A severely infected leaf of *P. imbricata*, showing spots in different stages of development.

are soon destroyed, however, and the spots become visible on both sides. On reaching a diameter of about 5 mm., a diseased spot assumes in most cases the characteristic appearance of a wide crater with the center depressed and the margin raised above that of the healthy tissues surrounding it. The diseased leaf finally turns brown and dries and the acervuli of the causal organism are evident in great abundance over the surface of the leaf. The crater-like spots above described, characteristic of the disease before the death of the leaf occurs, are still clearly visible even after the entire leaf blade is dead, but the acervuli are not confined to these spots.

The causal organism belongs to the form genus Gloeosporium. Preliminary infection experiments on apple resulted in the production of a

disease the symptoms of which were identical with those of typical bitter rot. The perfect stage of the organism has not been found, however, on the leaves of Pholidota, nor has it yet been induced in culture or on any of the hosts that have proved susceptible to the organism. It is, therefore, impossible to ascertain, as yet, whether this Gloeosporium is the imperfect stage of *Glomerella cingulata*.

Glomerella cingulata and several Gloeosporia have been reported in the literature on various orchids other than Pholidota, but in no case, as far as the writer is aware, have they been reported as causing serious damage. The fact that this disease may assume serious proportions, as in the case with the infected plants at the university greenhouses at Urbana, seemed to justify publishing this preliminary note concerning it. Studies of the causal organism in culture and on a number of susceptible hosts are being undertaken and will be reported later.—CONST. J. ALEXOPOULOS, Department of Botany, University of Illinois.

A New Canker of Hops in Oregon.[1]—An unreported canker of hops was observed in an experimental hop yard in Linn county, near Corvallis, Oregon, on June 19, 1934 (Fig. 1, A, B). Although many yards have been visited during the past 3 years, this abnormality has not been previously observed.

Rarely, leaf petioles, occasionally the tips, commonly the internodes of untrained shoots arising from the crowns of otherwise healthy plants were affected. Almost invariably, the cankers developed at the points of contact of the shoots with the soil, usually under exposure to sunshine but sometimes where shaded. The occurrence of the cankers did not appear to warrant their explanation as due to heat, despite the fact that yards on the sandier soils seemed most affected.

Affected portions of the vines at first develop yellow to light brown discolored areas that usually turn dark brown or almost jet black, involving the entire shoot for a distance of several inches. Particularly during periods of bright, hot days, the lesions often exude drops of clear exudate that soon become darker, more viscid, sometimes coalesce and turn a deep amber color when dry. Cankered areas completely wilt and dry up, causing death of the tip of the shoot beyond the canker.

Instances have been observed in which cankered areas turn light brown or straw color, wilt, and then shrivel without further color changes or development of exudate.

Smears of the exudate to agar slants have, in most instances, developed apparently pure cultures of bacteria.

[1] Published as Technical Paper No. 235, with the approval of the Director of the Oregon Agricultural Experiment Station.

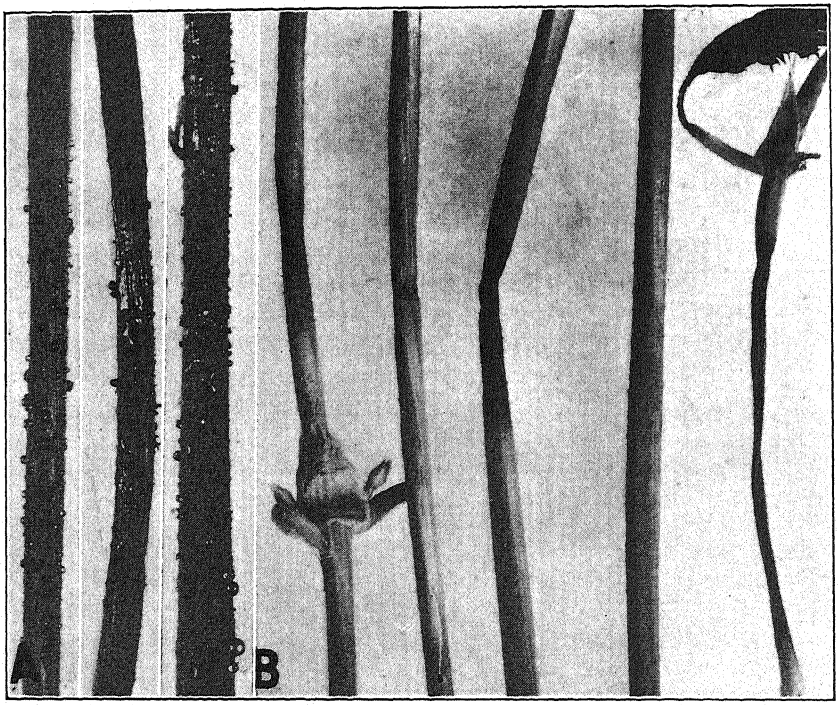


FIG. 1. Various stages in the development of hop canker. A. Drops of exudate containing bacteria. B. Discoloration and atrophy of cankered stems.

Sucking insects that might be responsible for the spread of the disease were not observed on any of the cankered shoots, but feeding scars of the 12-spotted leaf beetle, *Diobrotica soror,* were common in most of the affected plantings but rarely at cankered areas of the stems.

A field survey showed the canker present on the Early Clusters variety in 2 yards in Lane, 1 in Linn, and 3 in Marion counties; on Fuggles in 1 yard each in Marion, Polk and Yamhill counties; on Late Clusters in 1 yard in Benton, 1 in Lane, 4 in Linn, 4 in Polk, and 1 in Yamhill counties; on Red Vines in 1 yard in Linn, 2 in Marion, 2 in Polk, and 1 in Yamhill counties. The latest date on which the canker was found in the field was September 5.

Since early training of vines had been completed before the canker was observed, the economic importance of the injury was slight. Should this trouble appear previous to training, however, the damage to healthy vines might assume commercial significance.

A number of cultures of the bacteria obtained from canker exudate from different yards in various sections of the State are being maintained

in the hope of identifying the organism, conducting cultural studies, effecting artificial inoculations, and making histological examination of dismissed tissues.—G. R. HOERNER and D. C. SMITH,[2] Corvallis, Oregon.

Crown Gall on the Sequoia.—Crown gall on the conifers has only recently been reported in the literature. Brown and Evans[1] found crown gall to be of rare occurrence on *Cupressus arizonica* Green. Miles and Brown[2] list crown gall near the graft union of *Juniperus sabina* L. and Persons[3] reports gall on this same host. Patel,[4] in his agglutination studies, used a culture furnished by the writer from incense cedar, *Libocedrus decurrens* Torr. Muncie and Patel[5] list this same culture in table 7 of their paper. Hansen and Smith[6] studied a white nonmotile rod-like organism causing galls on the Douglas fir, *Pseudotsuga taxifolia* (Lamb.) Britt. The structure of the galls and the characteristics of the organism indicate a different causal organism from *Pseudomonas tumefaciens.*

In the spring of 1934 a culture of *Pseudomonas tumefaciens* isolated from a peach seedling was used in making inoculations on *Sequoia gigantea* (Lindl.) Dec. and *S. sempervirens* (Lamb.) Endl. Fifteen puncture inoculations were made in the open on each of the 2 species during May, June, and August.

One typical gall, $\frac{3}{4}$ of an inch in diameter, was observed on *Sequoia gigantea* in September about 4 months after inoculation; but the gall probably had appeared much earlier than this date. Another smaller hypertrophy, about $\frac{1}{8}$ of an inch in diameter, was observed in October. All the other wounds had healed with no indication of any hypertrophies. Figure 1, B, shows these healed-over wounds above and below the gall.

The inoculations on *Sequoia sempervirens* are negative after 4 months with 2 doubtful inoculations having small enlargements with knot-like outgrowths that may represent the beginnings of galls. The inoculations seem to suggest that sequoias are not immune from but somewhat resistant to

[2] Agents, Division of Drug and Related Plants, Bureau of Plant Industry, United States Department of Agriculture.

[1] Brown, J. G., and M. M. Evans. Crown gall on a conifer. Phytopath. 23: 97–101. 1933.

[2] U. S. Dept. Agr., Bur. Plant Industry, Pl. Dis. Repr. Sup. 81: 133. 1931.

[3] Persons, T. D. New or unusual diseases reported or observed in Mississippi in recent years. (Abst.) Phytopath. 24: 843. 1934.

[4] Patel, M. K. Some researches on *Pseudomonas tumefaciens.* (Abst.) Phytopath. 19: 98–99. 1929.

[5] Muncie, J. H., and M. K. Patel. Studies upon a bacteriophage specific for *Pseudomonas tumefaciens.* Phytopath. 20: 289–305. 1930.

[6] Hansen, H. N., and Ralph E. Smith. A bacterial gall disease of the Douglas fir. Science, 77: 628. 1933.

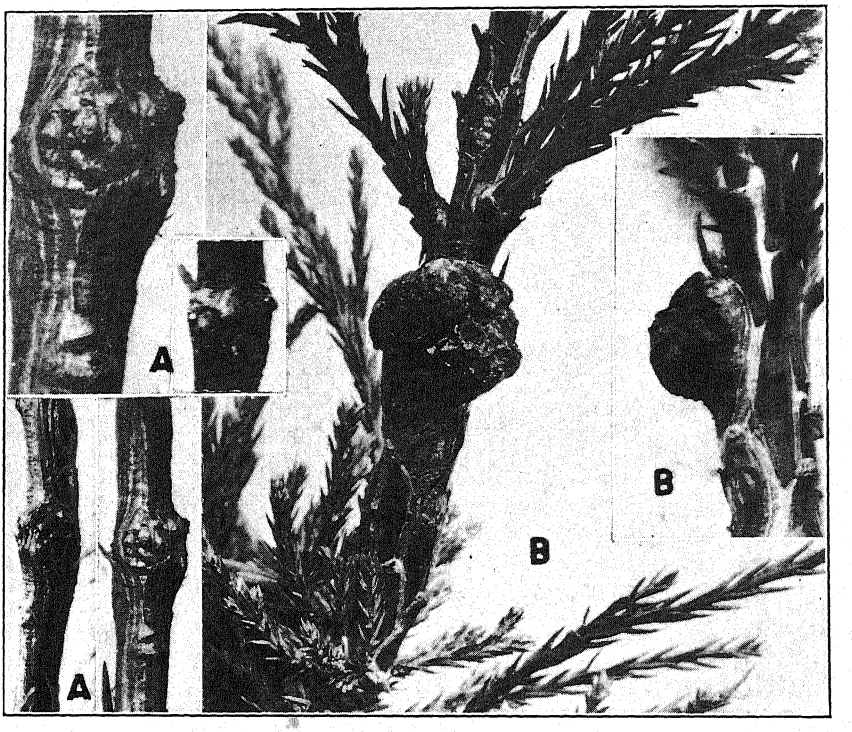


FIG. 1. Artificial inoculation on sequoias. A. Four different views of the same inoculation on redwood, *Sequoia sempervirens*, with a culture of *Pseudomonas tumefaciens* from peach made August 13, 1934, and photographed November 20, 1934. No typical galls have developed, but small knot-like elevations, which may be the initial galls, are present. B. Two views of a gall artificially produced on the big tree, *Sequoia gigantea*. Inoculations made on May 21, 1934, with a culture of *Pseudomonas tumefaciens* from peach. Photographed November 16, 1934. The scars above and below the gall represent inoculation wounds that have healed.

the crown-gall organism from peach. The gall development appears to be slower than that on peach and other deciduous trees.—CLAYTON O. SMITH, University of California, Citrus Experiment Station and Graduate School of Tropical Agriculture, Riverside, California.

The Growth of Agaricus Campestris on Plots Treated with Sodium Chlorate.—Early in August, 1934, mushrooms (*Agaricus campestris*) were noticed growing on plots that had been treated with sodium chlorate. Part of a field that was covered with quack grass had been laid out in plots 11.5 feet square. Alternate plots were treated with sodium chlorate at the rate of 1.5 pounds to a gallon of water on 100 square feet. This treatment was made on June 1, and was sufficient to kill all of the quack grass and other weeds that were growing on the areas treated.

TABLE 1.—*Mushrooms growing on plots treated with sodium chlorate and on adjoining nontreated plots*

August 3		August 16	
Treated	Nontreated	Treated	Nontreated
7	0	5	6
19	4	15	1
2	5	0	0
4	0	2	0
10	12	4	1
16	3	0	2
27	5	4	0
40	0	5	0
17	0	4	0
13	0	3	1
19	3	7	4
26	1	8	0
21	13	1	1
47	1	8	1
25	0	4	0
Totals 293	47	70	17

Two counts of the mushrooms on treated and nontreated plots were made (Table 1), one on August 3, the other on August 16. Thirty plots were used in each count, giving an area of 1984 square feet of treated soil and the same amount of untreated soil. At the time of the first count there were more than 6 times as many mushrooms on the treated as on the nontreated plots. The second count showed over 4 times as many mushrooms on the treated as on the nontreated plots. Most of the mushrooms on the treated plots were well scattered, whereas on the nontreated plots, most of them were near the sides bordering on the treated areas.

Two questions arose in connection with these observations: Did the mycelium develop in the presence of the sodium chlorate; and why were there so many more mushrooms on the treated plots? When mushroom beds are planted with actively growing mycelial cultures and maintained under favorable growing conditions they do not start producing mushrooms in less than 6 to 10 weeks. Aslander[1] found that there was still some sodium chlorate left in soil 10 weeks after it was applied. Since mushrooms appeared in less than 9 weeks it is obvious that dilute sodium chlorate is not toxic to *Agaricus campestris*. Species of Penicillium, Aspergillus, and Fusarium have been found growing on hay infusion treated with sodium chlorate.

There are several possible explanations for the increased production of

[1] Aslander, Alfred. Experiments on the eradication of Canada thistle, *Cirsium arvense*, with chlorates and other herbicides. Jour. Agr. Res. 36 (11): 915–934. 1928.

mushrooms on the treated areas. The most probable ones being lack of competition and an increase in available food materials. There is another possibility, however, that might be of importance, *i.e.*, a stimulation of growth due to the decomposition of the chlorate. Aslander quotes Alvisi and Orabona[2] as having found that *Penicillium glaucum* Link decomposed potassium and ammonium chlorates.—G. M. SHEAR, Virginia Agricultural Experiment Station, Blacksburg, Va.

Air Currents as a Possible Carrier of Ceratostomella ulmi.—Preliminary experiments indicate that spores of *Ceratostomella ulmi* (Schwarz) Buisman may be dislodged from mycelium or coremia by air currents. In a few tests in which cultures of the fungus were fanned for a few minutes it was found that *C. ulmi* developed subsequently on agar plates exposed for 2 hours at distances of 5 to 40 feet from the source culture.

This experiment was conducted in a tightly closed auditorium $30 \times 28 \times 14$ feet. Sterile agar plates were distributed uniformly throughout the auditorium after which a culture in one end of the room was fanned with a small piece of cardboard for five minutes. The sterile agar plates were exposed for 2 hours and then were incubated at room temperature for 3 days.

Three types of cultures were used, namely (1) a 5-day-old agar culture, (2) a 10-day-old culture on moist elm blocks with a small amount of mycelium and numerous coremia with glistening viscous heads, and (3) a

Date	Type and condition of culture	Source of spores	No. of plates exposed	No. of plates with *C. ulmi* after 3 days' incubation
1/2/35	5-day-old agar plate culture	Mycelium with conidia	60	60
1/22/35	Check	Room air fanned, no culture present	20	0
1/25/35	10-day-old fresh culture on moist elm blocks	Coremia and mycelium with conidia	60	23
1/30/35	Check elm blocks	Room air fanned, no culture present	20	0
2/2/35	24-day-old dried culture on dry elm blocks	Coremia and mycelium with conidia	60	13

[2] Alvisi, U., and M. Orabona. Sul comportamento di perclorati e clorati, nitrati e nitriti in alcuni esperimenti di chimica biologica e sul potere riducente de'Tubercoli radicoli delle Leguminose. Gaz. Chim. Ital. 42 (1): 565–575. 1912.

24-day-old culture on dry elm blocks also showing a small amount of mycelium and numerous coremia whose heads had dried to a gelatinous consistency.

The results of the examination of the exposed plates are given in the tabulation on page 442.

The results indicate that spores of the Dutch elm-disease fungus may be dislodged from fruiting structures on the surface of elm wood if exposed to air currents. Spores from the old dried culture apparently were dislodged less readily than those from the fresh young culture. The check plates exposed in the same room 2–3 days before the cultures were fanned remained sterile. Other experiments indicate that should viable spores lodge in a favorable place, such as in a fresh wound in an elm tree, infection may occur.—S. J. SMUCKER, Forest Pathology, Bureau of Plant Industry and Emergency Conservation Work.

Isolate vs. Isolant.—There seems to be some difference of opinion as to which of these words is preferable for use as a noun to indicate something isolated. Attention is called, therefore, to the fact that the word "isolate," not "isolant," is recognized in the latest edition of Webster's Dictionary in the sense referred to. It is the appropriate Anglicized form derived from *isolatus,* the past participle of *isolare* (= to isolate). "Isolant," on the other hand, would carry an entirely different meaning, being the natural Anglicized present principle form of the same verb. It would convey the transitive meaning isolating (= causing isolation) which, of course, could not rightly refer to an organism that has been isolated.

We have a parallel situation in the use of "precipitant" which the dictionary says means, "that which causes precipitation," and "precipitate" meaning "something that has been precipitated." Similarly, we have "filtrate" (= having been filtered), not "filtrant," and "delegate" (= having been delegated), not "delegant."

Perhaps the confusion in usage arises from an incorrectly assumed analogy to such words as "variant." Here, however, the present participle meaning is clearly appropriate (= that which varies), but in this case, the verb has an intransitive meaning while the verb "isolate" does not.

Preference for the word "isolant" has been based by a recent worker on the avoidance of confusion, by which he doubtless implies that the noun "isolate" might be confused with the verb "isolate." It seems doubtful, however, whether such confusion would often appear. There are many common instances in which the noun and verb are alike, but which we seldom find confusing; for example, the word "delegate" may again be cited along with "fly," "fish," or "fight." In view of the facts presented, it is believed that the apparently growing use of the word "isolant" should be discouraged.—H. P. BARSS, Office of Experiment Stations, U. S. Department of Agriculture, Washington, D. C.

Atanasoff, D. *Virus diseases of plants. A bibliography.* Houdojnik Printing Co., Sofia (Bulgaria), 1934. 219 pp. $3.00.

The author undertook a big task and has done it well. With close to 4,000 titles on a subject relatively so new to modern research methods as are the plant viruses, the omissions must be rare indeed. In fact, the greatest strength and value of the list comes from the inclusion of old or rarely mentioned titles of historical interest. The references are classified mainly on the basis of host plant affected and are further grouped according to plant family. While this arrangement may not be ideal, it is convenient, and its usefulness is enhanced by the addition of a host index as well as an author index. Papers dealing with more general or extensive subjects are listed in two additional introductory sections. The location of abstracts of original articles is also freely given, but no annotations are presented. In general, this compilation will prove invaluable to the virus research workers, especially so, since the author proposes to issue an annual supplement to the present bibliography.—JAMES JOHNSON.

Atanasoff, D. *Bolesti na kulturnitě rastenija.* (Diseases of cultivated plants). 626 pp. 221 figs. Imprimerie de la cour. Sofia. 1934.

This is the first college textbook on plant diseases that has appeared thus far in the Bulgarian language. Its most distinguishing features are the scholarly approach adopted in the treatment of the individual topics; the consideration shown for practical control measures as far as these are available; the emphasis on results of field trials; and the intimate treatment of the virus diseases, especially those of the grape and the potato. The book consists of 6 chapters. An introduction is followed by a chapter on each of the following: nonparasitic, bacterial, virus, and fungus diseases and diseases caused by seed plants. The book ends with a Bulgarian and a Latin index. Both in legends and in general text, frequent use is made of Latin names of host or parasite. These, with bibliographic citations, are useful indications to foreign readers as to content and nature of subject matter. The textbook naturally bears evidence of the author's early training in Germany and America and his later sojourn in Holland. The latter is shown by his treatment of the virus problems. His acquaintance with American literature is shown in literature citations and by the influence of the manuals of Heald and Elliott. Possibly, this text may invite increasing attention from neighboring countries because of the proximity of Bulgarian problems and their interpretation in some cases in a familiar language. This may, therefore, be accepted as a textbook notably and worthily broadening the field of influence of modern plant pathology.—S. S. IVANOFF, Department of Plant Pathology, University of Wisconsin, Madison, Wis.

THE AMERICAN PHYTOPATHOLOGICAL SOCIETY
CONSTITUTION[1]

ARTICLE I

This Society shall be known as The American Phytopathological Society.

ARTICLE II

MEMBERSHIP

Sec. 1. The Society shall consist of members, and may include life members and patrons.

Sec. 2. The charter membership of this Society shall consist of the one hundred and thirty persons who accepted the invitation of the Organization Committee of October 25, 1909, to form the Society.

ARTICLE III

QUALIFICATIONS FOR MEMBERSHIP AND DUES

Sec. 1. All persons interested in the study of phytopathology, including the practical control of plant diseases, shall be eligible to membership.

Sec. 2. Any member may become a life member by paying one hundred dollars in ten consecutive annual payments, and any person may become a patron upon the payment of one hundred dollars, and upon election shall have all the privileges of membership.

ARTICLE IV

ELECTION OF MEMBERS

Members may be elected at any regular meeting of the Society or by the Council during the interim. Applications for membership must be endorsed by at least one member of the Society.

ARTICLE V

OFFICERS

The officers of the Society shall consist of a President, Vice-President, Secretary, and Treasurer. Their duties shall be those usually performed by such officers. The President and Vice-President shall serve for one year and the Secretary for three years, or until their successors are elected, and the Council shall fill any vacancies occurring between annual meetings. The Treasurer shall be appointed by the Council for a term of three years and the Council shall fill any vacancy occurring between annual meetings.

The Council shall consist of the President, Vice-President, Secretary, Treasurer, the retiring President, and the Chairman of the Board of Editors of the Journal of the Society, with two members elected one each year, who shall serve for a term of two years, and one member elected annually by each Division. The term of service of a Council member from a Division shall commence at the end of the annual meeting of the Society next following his election by the Division.

All action of the Council or officers must be authorized or approved by the Society.

[1] At the 1934 annual meeting at Pittsburgh, the Secretary was directed to have the constitution of the Society, as revised at that meeting, printed and distributed to the members. The last printing of the constitution was in PHYTOPATHOLOGY, Vol. XX, No. 12, Dec., 1930.

ARTICLE VI

ELECTION OF OFFICERS

The Secretary shall send nomination ballots for officers to each member of the Society in time to allow all nominations to be returned not less than two months before the date of the annual meeting. The Council shall make nominations for any office when such nominations are wanting. The three candidates for each office receiving the highest number of nominating votes shall be placed upon a final ballot, which shall be sent to each member one month before the annual meeting, provided, however, that, if the same person be found to have received sufficient ballots to qualify for nomination to more than one office, his name shall be placed on the final ballot as a candidate only for the highest of these offices as indicated in their order in Article V. Votes shall be mailed to the Secretary and canvassed by the Council. A plurality vote shall elect.

ARTICLE VII

EDITORS, COMMITTEES, AND APPOINTMENTS

The Editors of the official organ of the Society shall be selected by the Council subject to the approval of the Society.

Temporary or standing committees may be appointed at the discretion of the Society.

Unless otherwise directed, the President shall appoint all temporary committees that are to serve during his administration and shall fill all vacancies on standing committees that may occur during his term of office.

ARTICLE VIII

MEETING

An annual meeting shall be held at such a time and place each year as the Council may select, and additional meetings, including special or local meetings, for the presentation of papers, may be arranged by the Council at its discretion.

ARTICLE IX

DIVISIONS

Branch organizations or units within the Society, known as Divisions, may be established on a geographical basis, provided formal application setting forth the reasons for the establishment of the Division is made to, and approved by, the parent Society.

ARTICLE X

AMENDMENTS

This Constitution may be amended at any annual meeting by a three-fourths majority of all the members voting, notice of the proposed amendment having been sent to all the members at least one month previous to the meeting.

STANDING RULES

The rules under which The American Phytopathological Society operates are as follows:

PHYTOPATHOLOGY

1. The official publication of the Society shall be PHYTOPATHOLOGY.
   a. *Officers.* The officers of PHYTOPATHOLOGY shall be an Editor in Chief, term three years; three Editors, terms three years; twelve Associate Editors,

terms three years, four selected each year; Business Manager, term three years; and Advertising Manager, term one year.

b. *Selection of Officers.* The Editor in Chief and the Business Manager shall be selected by the Council and approved by the Society. The Editors and the twelve Associate Editors shall be selected by the Council in consultation with the Editor in Chief and approved by the Society. The Advertising Manager shall be selected by the Council in consultation with the Business Manager and approved by the Society.

c. *Subscriptions, Back Numbers.* Subscriptions to PHYTOPATHOLOGY for institutions and nonmembers shall be $6 per year in the United States and dependencies; Canada $6.25; other countries $6.50. The price of current single numbers shall be 60 cents. The price of back volumes and numbers shall be determined by the business manager with the approval of the Council. Separate copies will not be sold except in cases where the volumes are already broken. Requests to supply lost copies of the journal without charge must be made within sixty days from date of issue.

DUES

2. The annual dues for each regular member, including subscription to PHYTOPATHOLOGY, shall be $5 per year, payable on December 20. The Business Manager of PHYTOPATHOLOGY shall discontinue sending the journal to any members whose dues have not been received by December 20.

PAPERS, ABSTRACTS

3. Members desiring to present papers at the annual meeting must furnish to the Secretary carefully prepared abstracts presenting as clearly and concisely as possible the substance and conclusions of the papers, these abstracts to embody definite results and not to exceed two hundred words in length.

a. *Date Due.* The Secretary is authorized to refuse abstracts received by him after the date on which they are due (Nov. 15). Members are requested not to submit titles or abstracts unless they intend to be present at the meetings.

b. *Number of.* No member shall be permitted to present more than two papers at any one meeting, except by invitation, and, in case of joint authorship, the paper shall be charged to the author presenting it.

c. *Editing of.* All abstracts shall be submitted to an editorial committee of at least three selected by the Editor in Chief of PHYTOPATHOLOGY, and this committee will edit the abstracts in the same manner as original articles published in PHYTOPATHOLOGY.

PROGRAMS

4. The program for the annual meeting shall be in charge of a program committee consisting of the President, Vice-President, and Secretary. To relieve congestion the program committee is authorized to schedule simultaneous sessions when necessary.

DIVISIONS

5. The following provisions shall govern the organization and regulation of Divisions of the Society.

a. *Name of.* Divisions shall use the name of the parent Society with the ap-

propriate geographical term, for example, The American Phytopathological Society, Pacific Division.

b. *Membership.* Divisions shall elect to full membership only members of The American Phytopathological Society, but each Division may elect associate members under such rules as it may adopt.

c. *Publication.* The proceedings of Divisions shall be printed in PHYTOPATHOLOGY. The preliminary abstracts of the Division meetings may, at the discretion of these Divisions, be printed in PHYTOPATHOLOGY under the same rules that govern publication of abstracts of the general Society. This rule, however, shall not be interpreted as limiting the present right of the Editorial Board of PHYTOPATHOLOGY to define the character and amount of any manuscript for publication.

d. *Meetings of.* Whenever The American Phytopathological Society meets within the territory of a Division, the Division shall merge its program with that of the parent society, The scientific sessions of such a meeting shall be presided over alternately by the President of The American Phytopathological Society and the President of the Division. Business sessions may be independent.

e. *Constitution of.* The constitution or articles of organization of all Divisions shall contain a provision or provisions ratifying the above rules. The constitution of all Divisions shall contain nothing in conflict with the constitution of The American Phytopathological Society. With the exceptions defined by the above rules, the Divisions shall enjoy complete autonomy.

6. Unless otherwise ordered, the Secretary and the President, or, in case of his inability to attend, the Vice-President, are authorized to attend the annual meetings of the Society at the Society's expense.

AUDITING COMMITTEE

7. At each annual meeting the President shall appoint an auditing committee to audit the accounts of the Treasurer of the Society and the Business Manager of PHYTOPATHOLOGY.

UNION OF BIOLOGICAL SOCIETIES

8. The Society shall participate in the work of the Union of American Biological Societies and designate its Secretary and Editor in Chief of PHYTOPATHOLOGY as its representatives unless otherwise voted.

BIOLOGICAL ABSTRACTS

9. The Society shall provide for a standing committee of five with terms of five years, one member being chosen each year, to cooperate with the Board of Editors of *Biological Abstracts.*

CROP PROTECTION INSTITUTE

10. Representation on the Board of Governors of the Crop Protection Institute shall be provided for by three trustees, with three-year terms, one selected each year, these trustees to be chosen by the Council.

NATIONAL RESEARCH COUNCIL

11. Representation on the Division of Biology and Agriculture of the National Research Council is provided for in the following way. The various societies represented in the Division are classified into groups. This Society and the Society of American Bacteriologists constitute Group V. This group, as is the case with the others, likewise is entitled to one representative. Each Society shall designate an elector and an alternate for three-year terms. The two electors are to choose the representative on the Division, for Group V, either one of themselves, or a member of one of the societies, the societies being taken in rotation. The term of the representative shall be three years. The elector from the Society not having the representative on the Division in a given period, or some other member whom the Society may select, shall serve as an advisory representative without vote, and without expenses paid by the National Research Council, and may attend divisional meetings to present directly any business of his society.

The elector and alternate of The American Phytopathological Society, terms three years, shall be selected by the Council of the Society and approved by the Society.

OTHER REPRESENTATIVES

12. The following shall be selected by the Council and approved by the Society: two representatives on the Council of American Association for the Advancement of Science for one-year term; one trustee, on the Tropical Plant Research Foundation for a five-year term, and one member of the Editorial Board of the American Journal of Botany for a three-year term.

AMENDMENTS

13. These rules may be amended by a majority vote of the members voting at any regular meeting of the Society.

ANNOUNCEMENT

Members and advertisers are asked to note the new addresses to which all business and other communications relating to the Society and its journal should now be directed:

Secretary—H. P. BARSS, Office of Experiment Stations, U. S. D. A., Washington, D. C.

Treasurer and Business Manager of PHYTOPATHOLOGY—H. H. EDSON, Plant Disease Survey, Bureau of Plant Industry, Washington, D. C.

Advertising Manager of PHYTOPATHOLOGY—R. S. KIRBY, Pennsylvania State College, State College, Pennsylvania.

NOTES AND NOTICES, AMERICAN PHYTOPATHOLOGICAL SOCIETY

A. A. A. S. Entrance Fee Waived. The American Association for the Advancement of Science calls attention to the fact that by reason of the affiliation of our Society any member is privileged to join the Association by paying only the annual dues of $5.00, the usual entrance fee of $5.00 being waived. Apply to the Permanent Secretary, A. A. A. S., Smithsonian Institution, Washington, D. C.

Sixth International Botanical Congress will be held Sept. 2–7, 1935, at Amsterdam. Dr. Johanna Westerdijk, Baarn, Netherlands, is chairman of the Section for Phytopathology.

Any person interested in botany may become a member of the Congress on payment of the subscription of 1250 guilders and will be admitted to all Congress meetings and functions. The preliminary program may be obtained from Dr. M. J. Sirks, Secretary of the Congress, Wageningen, Netherlands.

Any member of the Society expecting to attend this Congress should immediately notify the Secretary of the Society. A good representation is expected.

The International Union of Biological Sciences, it is reported, will meet just prior to the Botanical Congress, to consider amendments to the constitution proposed by the Botanical Section. These would permit the affilia-

tion of individual scientific societies and would clear the way for the Council of this Society to arrange for its adhesion as directed by the Annual Meeting.

Summer Meeting. The Council has authorized the holding of a Summer Meeting of the Society with Dr. E. C. Stakman in charge of arrangements. It will coincide with the Summer Meeting of the A. A. A. S., which is to be held at Minneapolis, June 24–29, 1935. The members will receive further notification regarding program and arrangements.

Former Members may be restored to Active Membership at any time by applying to the Secretary and sending in a check for $5.00 in favor of the Society. All issues of PHYTOPATHOLOGY for the current year will be sent.

H. P. BARSS, *Secretary,*
Office of Experiment Stations,
U. S. Department of Agriculture,
Washington, D. C.

SUMMER MEETING OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

The summer meeting of The American Phytopathological Society will be held at University Farm, St. Paul, Minnesota, from June 25 to June 27, inclusive. Headquarters will be in Room 107, Plant Pathology Building. Except for one symposium, there will be no formal papers. It is planned to devote considerable time to field trips and round-table discussions at which everyone may express himself as freely as he wishes on any subject related to phytopathology.

Some of the general topics suggested for discussion are as follows:

1. The responsibility of plant pathologists in planning problems of regional or national scope.
2. Regional cooperation on plant pathological problems.
3. Problems in teaching plant pathology to students and to farmers.
4. Improving facilities for publication.

In addition, it is hoped to have discussions on many other topics. The fullest opportunity will be given for exchange of views.

The tentative program is as follows:

June 25—9:00 A. M.

Joint session with Section O of the A. A. A. S., Dairy Science Association, Corn Belt Section of The American Society of Agronomy, American Society of Horticultural Science, Great Plains Section, and The American Society of Plant Physiologists. The general topic will be, "Improving the Germ Plasm of Domestic Plants and Animals." Auditorium of the Administration Building, University Farm.

2:00 P. M.
Symposium, "Past, Present, and Future of Phytopathology in North America." Room 107, Plant Pathology Building, University Farm. To be followed by general discussion.

The evening will be left free for informal round-table discussions or attendance at the general meeting of the A. A. A. S.

June 26—Visits to field plots of the Divisions of Plant Pathology and Botany and Agronomy and Plant Genetics; laboratory and greenhouse exhibits.

In the evening there will be an informal dinner at University Farm, to be followed by round-table discussions.

June 27—Field trips to vegetable plots near the Twin Cities, to the State Fruit Breeding Farm, and to sugar-beet experimental plots at Chaska.

Every effort will be made to provide for the fullest and freest exchange of views. Formality will be dispensed with; the meeting is designed to give everyone opportunity to give and to receive information and ideas.

It is suggested that hotel reservations be made soon, as there is likely to be a fairly large attendance at the A. A. A. S. meetings. Almost any type of accommodation can be obtained at the following Minneapolis hotels:

Name	*Address*	*Without Bath*		*With Bath*	
		Single	*Double*	*Single*	*Double*
Curtis Hotel	10th and 3rd to 4th Ave. S.			$2.00 up	$3.00 up
Nicollet Hotel	Washington and Nicollet	$2.00	$3.50	2.50 up	4.00 up
West Hotel	Hennepin at 5th Street	1.25 up	2.00 up	1.75 up	2.50 up
Andrews Hotel	Hennepin at 4th Street	1.50	2.50	2.25 up	3.25 up
Hotel Vendome	17 S., 4th Street	1.00 up	1.50 up	2.50 up	2.50 up

A list of private rooms near University Farm will be available in Room 107, Plant Pathology Building, in case any members wish to obtain accommodations in private houses.

PHYTOPATHOLOGY

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AECIOSPORE INFECTION IN GYMNOCONIA INTERSTITIALIS BY PENETRATION OF THE CUTICLE[1]

S. M. PADY[2]

(Accepted for publication November 24, 1934)

It is commonly accepted as a fact that aeciospore germ tubes of the rusts normally enter their hosts through the stomatal opening. A survey of the literature reveals that no exceptions to this general rule are known. Recently, however, the writer discovered that when aeciospores of *Gymnoconia interstitialis* (Schl.) Lagerh., the orange rust of Rubus, were sown upon healthy leaves the germ tubes were able to penetrate the epidermis at any point, irrespective of the presence or absence of stomata. This was accomplished by means of enlarged spore-like bodies, the appressoria, formed at the ends of the germ tubes. Since this type of penetration heretofore has not been reported, detailed series of inoculation experiments were undertaken. This communication gives the results of these inoculation studies as well as a detailed description of penetration and the establishment of infection, and at the same time throws more light upon the host-parasite relationship in the rusts.

The first record of the method by which the germ tubes of aeciospores effect an entrance into their hosts is found in the classical researches of de Bary (9) in 1863. He inoculated leaves with aeciospores of many species and observed that the germ tubes always entered through the stomatal opening. Urediospore germ tubes behaved in a similar fashion. Even when inoculations were made on hosts other than their own there was no difference in the type of penetration. Gibson (25) performed similar infection experiments with 18 different species using urediospores in 12 species and aeciospores in the remainder. Her results are similar to those of de Bary. Clinton and McCormick (17) found germ tubes of aeciospores of *Cronartium ribicola* entering the stomata of leaves of *Ribes* sp. Zeller and Lund (39) observed that in *Phragmidium rubi-idaei* aeciospore and

[1] This work was done during the tenure of a National Research Fellowship in Botany at the New York Botanical Garden, New York.

[2] The writer wishes to acknowledge his indebtedness to Dr. B. O. Dodge and to Dr. R. A. Harper for their valuable advice and criticism during the course of the investigation.

urediospore infection always took place through the stomata. Ashworth (7) has recently reported a similar method of infection by the aeciospore germ tubes of *Coleosporium tussilaginis.*

Clinton (16) was the first to make inoculations with the long-cycle strain of Gymnoconia. Aeciospores were placed on leaves in Petri dishes on June 11, and on July 14 teliospores were observed. He concluded that "infection from the aecial spores of Gymnoconia takes place only through the stomates of the leaves and the telial stage results from this."

In 1923 Dodge (20) successfully inoculated cultivated blackberry varieties with Gymnoconia aeciospores obtained from *Rubus canadensis* and also obtained teliospores on the leaves of both blackberries and dewberries by sowing aeciospores from the black raspberry. This latter host was in turn infected with aeciospores from the blackberry. Dodge, in a later paper (21), while studying the development and distribution of the stomata on infected leaves, came to the conclusion that these leaves bore teliospores earlier and more abundantly than normal leaves, and that "the ease with which they may become infected is, at least in part, due to accessory dorsal stomata and freedom from tomentum." Aeciospores were sown on leaves, and after 2 days in a moist chamber, the leaves were dropped in Flemming's fixative. After fixation the chlorophyll was removed and they were mounted on a slide and examined. Dodge reported that "the germ tube grows along the surface until it comes into the immediate vicinity of a stoma, then, if necessary, the end turns sharply, broadens out and sends an infection tube through the opening."

The so-called "abnormal" germination, in which the contents of the spore and germ tube move into a "secondary body" or vesicle has been reported by many authors. These structures have been observed on the germ tubes of aeciospores by Tulasne (35) for *Aecidium violae* and *Roestelia cancellata,* by Sappin-Trouffy (31) for *Uromyces erythronii, Puccinia rubigo-vera, Roestelia lacerata* and *Peridermium pini,* and by Dodge (19) for *Gymnosporangium transformans.* Similar bodies have been noted on urediospore germ tubes of *Cronartium asclepiadum* (Tulasne (35)), *Puccinia graminis* (Plowright (28) and Sappin-Trouffy (31)), *P. iridis, P. fagopyri* (Barclay (8)), *P. Polygonii, Phragmidium rubi, Cronartium flaccidum* (Sappin-Trouffy (31)), and *Cronartium ribicola* (Spaulding (33)). In all cases the spores were sown on water and their behavior on leaves is unknown.

PRELIMINARY STUDIES

In January the writer had a number of infected blackberry plants growing in the greenhouse. Germination tests were made on water and on 2 per cent agar as the aecia matured, as it was not known definitely whether

these plants bore the long-cycle or short-cycle strain. Although only long germ tubes were formed, it was decided to study their behavior on leaves, as it is known that the aeciospores of the short-cycle strain sometimes produce long germ tubes Dodge (22).

Aeciospores were dusted over the lower surface of freshly washed leaves, placed in Petri dishes and left on a desk in the laboratory. A little water was added to the plate to maintain the humidity necessary for germination and care was exercised to avoid contact with the leaf. Small portions of the leaves were examined from time to time. The pieces were prepared for examination by first putting them in 95 per cent alcohol and boiling them until they had lost their chlorophyll. They were then transferred to lactophenol and boiled until the leaves were cleared. A very small amount of cotton blue usually was added to the lactophenol.

At the end of 24 hours the aeciospores had germinated, but instead of long germ tubes being produced the entire contents had become concentrated in a rounded body at the end of a short germ tube. Although stomata were present in abundance, not a single germ tube could be found entering them. The next examination, 6 hours later, disclosed a short intracellular hyphal strand in the epidermis immediately beneath this terminal body, henceforth to be referred to as the appressorium. In view of the results of Clinton (16) and Dodge (21), who both state that infection takes place through the stomata, this behavior was somewhat surprising. It was decided, therefore, to make a series of inoculations in order to obtain information on the aeciospore infection of *Gymnoconia interstitialis*.

INOCULATION EXPERIMENTS

To obtain a generous supply of aeciospores, infected plants from different localities in the vicinity of New York were brought into the greenhouse and potted. Since the infected plants form witches' brooms, it is possible to recognize them even before the leaves appear. From a clump of blackberries known to be free from the rust, several plants were simultaneously potted, at the Botanical Garden, to ensure a supply of known healthy leaves. Aecia began to appear in about 30 days from the time of the planting and, as soon as the pustules had broken open, the aeciospores were used for inoculations. All inoculations were made in Petri dishes, as described above. The leaves were examined periodically and kept alive as long as possible. The cultures could be kept for a limited period only, because the leaves were not supplied with nutriment. In one instance, that of March 16, 3 cultures were maintained for a period of 25 days. During late May and early June the warm weather made it very difficult to keep cultures more than from 10 to 14 days. This, however, was sufficient time in which to determine whether or not infection had become established, for it had

been found in earlier experiments that the mycelium was abundant by the end of the third day.

As both the long-cycle and short-cycle rusts occur on blackberries, it is not possible to tell at once which strain has been collected. Accordingly, when the aecia matured, germination tests were made with spores from all plants. Of the total number of infected wild blackberries brought into the greenhouse, approximately one half proved to be infected with the long-cycle strain of the organism, indicating, therefore, that this strain is of common occurrence about New York City. Healthy and diseased roots of black raspberry, *Rubus occidentalis,* were collected at Tuckahoe, N. Y. The long-cycle rust is the only strain known for this host.

The results of inoculations from the rust on blackberries and black raspberries are summarized in tables 1 and 2. In the tables there is a natural division between those rusts that were preseason, that is, those forced in the greenhouse, and those that matured normally. A plus (+) sign indicates that penetration took place through the epidermis by means of appressoria. A negative (−) sign signifies no germination. The number in brackets following the date of the experiment indicates the number of the particular plant from which the inoculum was obtained.

On April 9 the leaves of two twigs were inoculated by spraying the lower surfaces of those of one twig and the upper surfaces of those of the other with a spore suspension from a rusted blackberry. Both twigs were

TABLE 1.—*Inoculations of Gymnoconia interstitialis on blackberry*

	Healthy leaves			Infected leaves	
	Blackberry		Black raspberry	Blackberry	Black raspberry
	Upper surface	Lower surface	Upper surface only		
Gymnoconia from greenhouse	+Mar. 16 (1) +Mar. 20 (6)[a] +Apr. 9 (3) −Apr. 11 (3) +Apr. 21 (27) +May 9 (62)	+Mar. 12 (1) +Apr. 9 (3)[b] +Apr. 24 (27) +May 10 (62)	+May 4 (27) +May 10 (62)	+May 17 (62)	−May 17 (62)

	Blackberry	Dewberry
Gymnoconia from field	+May 20 (Bot. Garden, N. Y.) +May 28 (West Virginia) +June 5 (Hunters Is., N. Y.)	+June 6 (Hunters Is., N. Y.)

+ = Successful penetration by appressoria.

− = Negative result (*i.e.* no germination).

[a] Seedlings of *Rubus canadensis.*

[b] Leaves on twigs.

kept in a large glass moist chamber. Aeciospores germinated readily and penetration took place from the appressoria at the ends of the germ tubes. This experiment confirmed earlier observations that penetration takes place as readily on the upper (stoma-free) surface as on the lower (stoma-bearing) surface.

The inoculations of the next series, April 21 and 24, were made to determine the effect of various external factors upon penetration. One set was left on the laboratory desk at room temperature; a second was similar but kept in the dark; a third on the cement floor; a fourth outside on the window sill; a fifth on a shelf in the darkened storeroom; a sixth in an oven at 25° C.; a seventh in the oven at 30° C.; and an eighth in the greenhouse, where the temperature was high in the daytime and low at night. The general result was that in all cases penetration took place. In the third set no germination had taken place and the leaf was completely dried up. There was no difference between those kept in the dark and those in the light. In the set kept outside where the temperature ranged from 6° to 18° C. during the course of the experiment, penetration was equally as good as in the case of the checks kept at room temperature. At 25°, germination was very slow and development was greatly retarded. There was no penetration at 30° and there were many irregularities and peculiarities in the germ tubes. Low temperatures apparently favor penetration and further development, while high temperatures retard germination and penetration and result in many irregularities. If the room temperature was high at the time of inoculation, the percentage of germination was usually lower.

A series of inoculations was made on infected leaves, the spores being dusted on the upper surface. The leaves bore the systemic mycelium and mature pycnia were abundant. As will be noted in the two columns at the right in table 1, penetration takes place readily on infected blackberries, and was independent of the stomata, which are abundant on the upper surface. In the series on black raspberries conditions were apparently not favorable for germination since spores from the same plant sown at the same time on blackberry leaves germinated readily.

When Gymnoconia matured in the field (see Tables) several series of inoculations were made using healthy blackberry leaves gathered at the same time. From table 1 it will be noted that the results were positive for all inoculations. In one experiment healthy dewberry leaves were substituted for those of the blackberry. Penetration was accomplished here also with equal facility.

In table 2 inoculations with aeciospores from black raspberry have been summarized. These results agree very closely with those in table 1 in that there is successful penetration of both the upper and lower surfaces

TABLE 2.—*Inoculations of Gymnoconia interstitialis on black raspberry (Rubus occidentalis)*

	Healthy leaves				Infected leaves
	Black raspberry		Blackberry		Black raspberry
	Upper surface	Lower surface	Upper surface	Lower surface	
Gymnoconia from greenhouse	+May 7 (57) +May 8 (58) +May 10 (57) +May 11 (49)	+May 14 (49)	−May 5 (57) +May 8 (57)	+May 7 (57) +May 14 (49)	+May 15 (49) +May 17 (49)[a] +May 19 (50)[a]
Gymnoconia from field					+June 14 Tuckahoe, N. Y.

+ = Successful penetration.

− = Negative result (*i.e.* no germination).

[a] Leaves had infection only on one half of leaf.

of healthy and infected leaves of both blackberries and black raspberries. In the one negative case of May 5, there was no germination, probably because this set of Petri dishes had been accidentally left in the sunlight and the resulting high temperature had inhibited all germination.

As a final check, partially infected leaves were inoculated (May 17, May 19). Since the infected portion of the leaf will bear stomata on its upper surface while the remainder of the leaf will be stoma-free (21) the dusting of aeciospores over the entire leaf should prove conclusively whether or not the stomata play any rôle in the act of penetration. Careful examination of the leaves showed that penetration by means of appressoria occurs over the entire area of the leaf.

In all of these inoculations penetration was never found to occur through the stomata. Although occasionally a few very long germ tubes are formed on the leaf, as for instance when the cultures are kept at 30° C., their growth could not be correlated in any way with the presence of adjacent stomata. Several cases were recorded where the germ tubes grew over the actual openings of the stomata, yet did not enter. When spores were placed on the lower surface of healthy leaves and also on the upper surface of infected ones, there was ample opportunity for stomatal penetration. As shown in the above tables, penetration takes place just as readily on the upper as on the lower side, irrespective of whether the host be blackberry, black raspberry, or dewberry or whether it be infected or not.

Details of Penetration

Within 24 hours from the time of inoculation the appressoria are fully formed. In shape they are, in general, similar to the aeciospore, *i.e.*,

globoid or ellipsoid, though they are slightly smaller (Fig. 1). The appressoria may be formed at the end of a long germ tube or, on the other hand, may be so close to the spore that it is almost impossible to find the germ tube. Their dense contents and thin wall are in striking contrast to the empty aeciospore with its thickened wall and verrucose markings. Their development is illustrated in Plate I, 1–3. From one of the germ pores in the spore a germ tube grows out and its tip begins to enlarge (Pl. I, 1). As the contents move toward the tip the aeciospore is gradually emptied and the appressorium becomes more and more dense (Pl. I, 1–3). A wall is laid down and the terminal appressorial cell is cut off (Pl. I, 3).

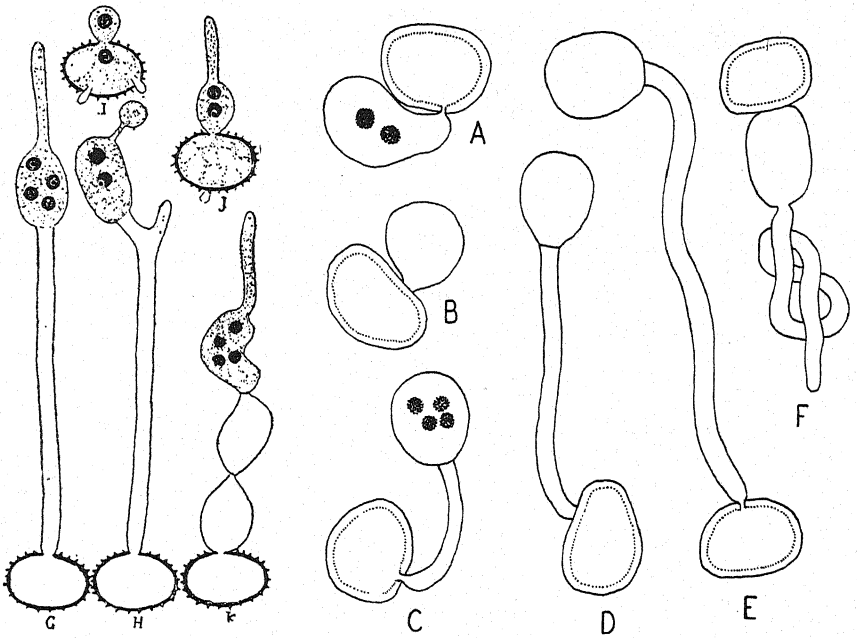


FIG. 1. At left, vesicles on aeciospore germ tubes of *Uromyces erythronii* (after Sappin-Trouffy (31) 1896). A–F. *Gymnoconia interstitialis*. A–D. Appressoria on leaves (24 hours). E. Appressorium formed at end of germ tube on agar. F. Appressorium that germinated but did not penetrate. ×560.

In the majority of cases the appressorium lies directly below the aeciospore, that is, between it and the surface of the leaf (Pl. I, 8, 9). This renders it very difficult to see their connection. Where the spore lies to one side, the connecting germ tube is readily observed (Pl. I, 4, 5, 7). Sometimes germ tubes reach a considerable length. This is especially true where there are hairs on the leaf. The spores are caught in the hairs and when germ tubes are formed they grow downward until they reach the leaf surface, where they form the appressoria. On the other hand, the

spores that fall directly on the leaf form appressoria immediately beneath them, or to one side, at the end of very short germ tubes.

The leaves of the black raspberry are strongly tomentose on the lower surface. Spores dusted on this surface fall among the hairs and long germ tubes are produced. The dense tomentum prevents one from readily following the entire length of the germ tubes, but if the leaf be well cleared in lactophenol, it may be turned over and examined from the upper surface. Where the germ tubes have reached the epidermis, appressoria can readily be found.

The two spore nuclei move out into the appressorium (Pl. I, 1) and sometimes divide, even before the septum has been laid down (Pl. I, 2). In this figure the position of the two pairs of nuclei suggests that conjugate division has just been completed. The mature appressorium is regularly 4-nucleate (Pl. I, 3, 4). At this stage they closely resemble the vesicles found by Sappin-Trouffy (31) on the aeciospore germ tubes of *Uromyces erythronii*. This similarity is at once apparent in figure 1 in which some of his illustrations have been reproduced for comparison with the appressoria of Gymnoconia (Fig. 1, A–F).

The appressorium flattens out against the epidermis (Fig. 2, A) and a penetration peg is formed. From above, the opening appears as a small white spot surrounded by a characteristic and slightly darker zone (Pl. I, 4, c). From this short penetration peg a penetration hypha is formed in the epidermal cell. Plate I, 4 shows a very young penetration hypha, while Plate I, 5 represents a slightly later stage. The cell contents pass in and the oil globules render it very distinct. Growth in the epidermis is rather limited, the hypha growing along the floor of the cell for a short distance; it then passes through the cell wall into the intercellular spaces below. This is well illustrated in figure 2, B, a cross section through an inoculated leaf. The aeciospore became detached during the preparation of the slide. The appressorium, however, remained attached to the leaf and the penetration hypha is a characteristic one.

The beginning of subepidermal or intercellular mycelium is clearly marked in the preparations made during the second and third days. In order to see this mycelium it is necessary to focus downward in the leaf tissue, indicating a change in level. This is best shown in cross section (Fig. 2). In plate I, 6, 53 hours after inoculation, a short hypha is seen growing away from the end of the penetration hypha and making its way deeper into the tissues, as indicated by the darker shading. The opening in the cell wall through which the contents pass is clearly seen in cross section (Fig. 2, B, C). The hypha in the latter figure has already branched and the two branches are following intercellular spaces in the palisade to the mesophyll below. This type of growth usually follows inoculation of

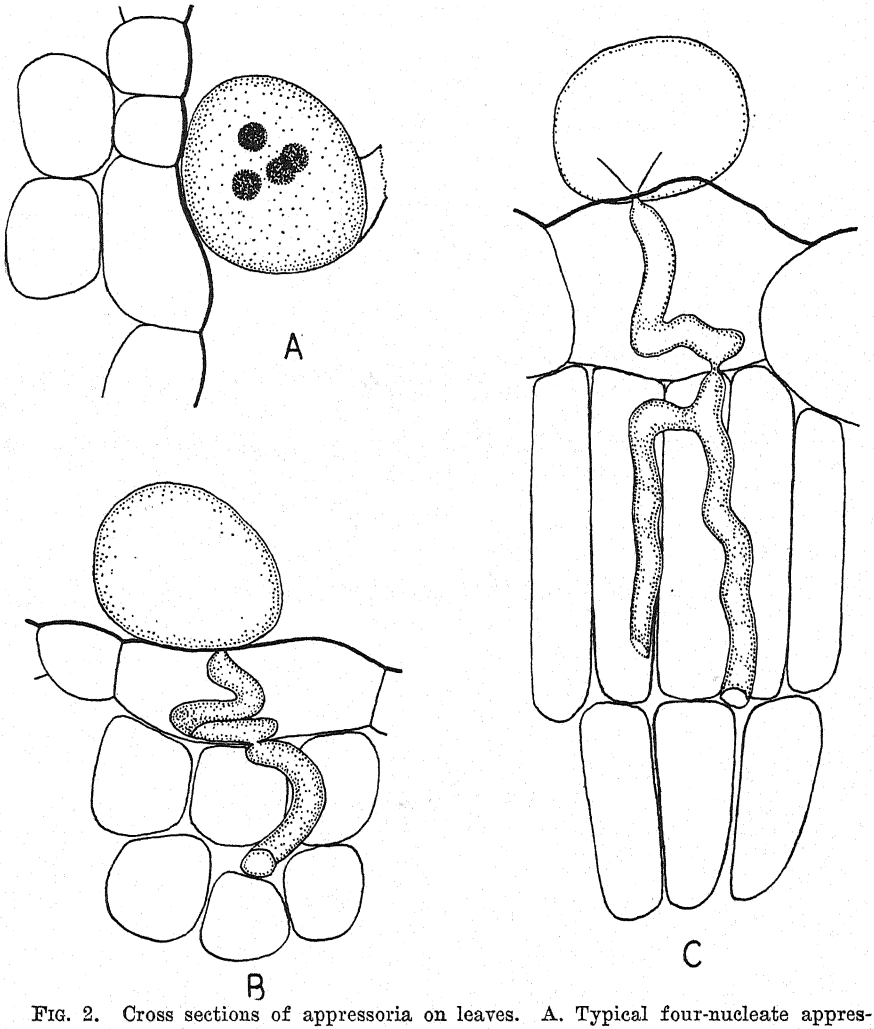


FIG. 2. Cross sections of appressoria on leaves. A. Typical four-nucleate appressorium. B. Penetration on lower epidermis. C. Well-advanced infection on upper epidermis. ×1240.

the upper surface. When the lower surface is inoculated the mycelium spreads near the leaf surface in the large intercellular spaces of the mesophyll.

Plate I, 7 is drawn from an inoculation on infected black raspberry. Although both the spore and the appressorium are partially lying over a stoma, it is at once apparent that penetration is entirely independent of them. The penetration hypha is short and the subepidermal hypha is dipping downward in the palisade tissue. In these infected leaves the haploid

mycelium is very abundant and haustoria are often present in the epidermal cell when the germinating aeciospore penetrates it (Pl. I, 8). Here, 53 hours after inoculation, the penetration hypha has branched and is giving rise to 2 hyphae in the intercellular spaces below. The hypha (f) at the right is growing toward the mesophyll between the palisade cells, faintly outlined in the drawing. The infected leaves were covered with pyncia and one case was observed where penetration had taken place directly on the base of the pycnium (Pl. II, 10). The host cell, already penetrated, contained 2 or 3 haustoria.

That the stomata play no rôle in penetration by germinating aeciospores is well illustrated in plate I, 9. The appressorium is typically beneath the empty aeciospore, and the penetration peg, 70 hours after inoculation, has entered the guard cell of a stoma. The penetration hypha is short and the subepidermal mycelium is extensive. There is a second hypha, which suggests that the appressorium has given rise to 2 penetration hyphae. It is quite possible, however, that this hypha may have come from a branch of the other subepidermal mycelium. The overlying guard cells, appressorium, and aeciospore make it very difficult to observe clearly the exact details.

The mycelium grows rapidly in the large intercellular spaces of the spongy mesophyll. Even at the end of the second day there may be considerable mycelium (Pl. II, 11). This usually is branched and the oil globules are very prominent. If a stoma is in the vicinity a hyphal branch usually enters the substomatal cavity (Pl. II, 12, 14). This is well-marked in infections of a week or more, the strands often running more or less directly from one substomatal cavity to another.

At the end of the fourth day the mycelium is fairly extensive (Pl. II, 13). There is no indication here of haustorium formation. The mycelium is beginning to outgrow the oil globules, and, while it is impossible to see nuclei along the course of the hyphae, at the tips the 2 nuclei of the dikaryon are readily observed. This figure also illustrates penetration at the extreme edge of the leaf. Many spores were found penetrating here, and the connection between the spore, appressorium, and penetration hypha may be clearly demonstrated. For the sake of clarity the cells of the leaf edge have been omitted from the drawing.

At the end of the fifth day haustoria begin to develop at various points on the mycelium. The epidermal cells are an excellent place in which to study their development, and all the drawings in figure 3, with the one exception of D, were taken from that part of the leaf. The haustorium mother cell usually is a short lateral branch of a main hypha (Fig. 3, E) and is regularly binucleate. If the mother cell is at the tip, the hyphal cell below continues to grow after the haustorium is formed (Fig. 3, F). The penetration peg is quite long and very slender (Fig. 3, A and B) and enters

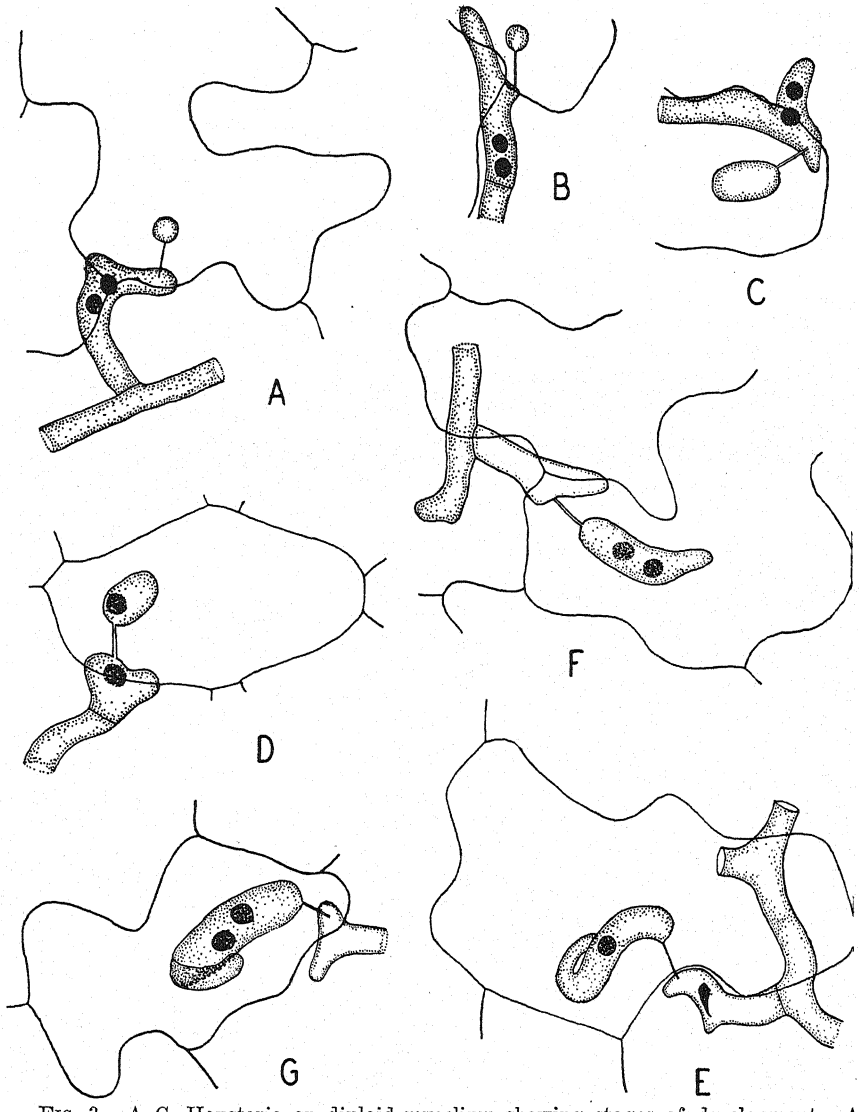


FIG. 3. A–G. Haustoria on diploid mycelium showing stages of development. All figures drawn from epidermal cells except D, which is in the mesophyll. ×1300.

the cell at a point near the side wall. At the tip a small round sac or vesicle appears (Fig. 3, A and B). This elongates as the contents of the mother cell move in (Fig. 3, C). One nucleus moves in (Fig. 3, D, E) and after a brief interval the other follows (Fig. 3, F). The mature haustorium is shown in figure 3, G. In older preparations it often is branched and the narrow tips lengthen, usually winding about the rest of the haustorium.

From this time on, the mycelium spreads rapidly through the leaf, par-

ticularly in the spongy mesophyll, forming haustoria in all tissues. The development of the mycelium, as well as the method of penetration, is well illustrated in the diagram in figure 4. Plate II, 14 was drawn from an 11-day-old preparation. Here, the mycelium was so abundant that only a small part of it could be included in the drawing. This case is remarkable in that no appressorium was formed, penetration taking place directly from the germ pore.

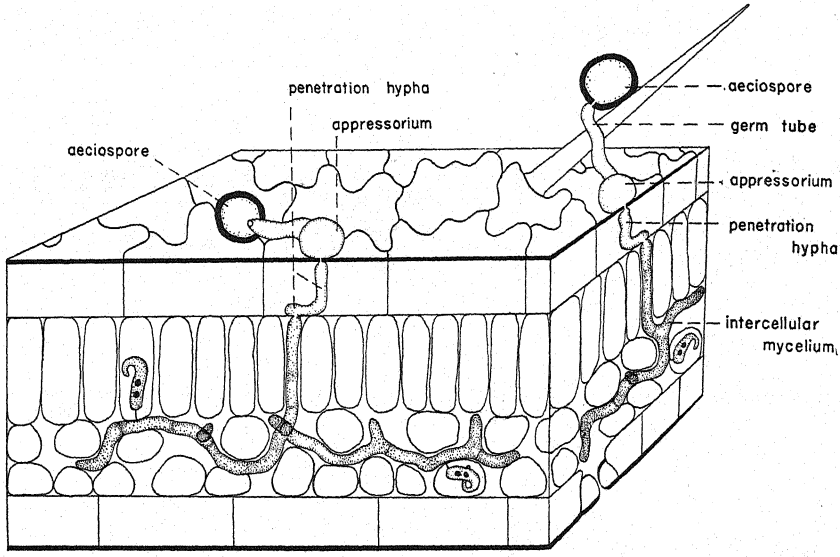


FIG. 4. Diagram of aeciospore infection in *Gymnoconia interstitialis*.

Teliospore Production

At the end of 21 days teliospores were formed in the series of March 16 (Table 1). The leaves in this case were inoculated on the upper surface and the culture was kept for 25 days. By this time small black telia were abundant on the lower surface. Since these teliospores were produced in Petri-dish cultures under very artificial conditions, 2 seedlings were inoculated in the greenhouse as a check (Table 1). These seedlings were of *Rubus canadensis* and were about 3 inches high when inoculated. After being kept 4 days in a moist chamber they were placed on the greenhouse bench. Teliospores appeared on the 28th day on the lower leaves, none being found on the upper leaves. These results agree very well with those of Clinton (16) and of Dodge (20).

In the greenhouse, at this time, were several infected plants bearing both the long-cycle and short-cycle rusts. The short-cycle rusts had completed their sporulation period by the time the long-cycle ones had matured. About a month later the older leaves of the plants were found to bear teliospores, thus indicating that penetration takes place readily on the older

leaves under ordinary greenhouse conditions. In order to see whether or not infection takes place by appressoria in nature, field collections of leaves bearing Gymnoconia telia were examined. This material was collected by B. O. Dodge at Inlet, N. Y., on August 23. The basal leaves of the canes were thickly dotted with the telia (Fig. 5). When small portions of the leaves were cleared in lactophenol and examined, empty appressoria were found on the upper surface, and even in some cases the penetration hypha could be discerned. No appressoria were found on the lower surface.

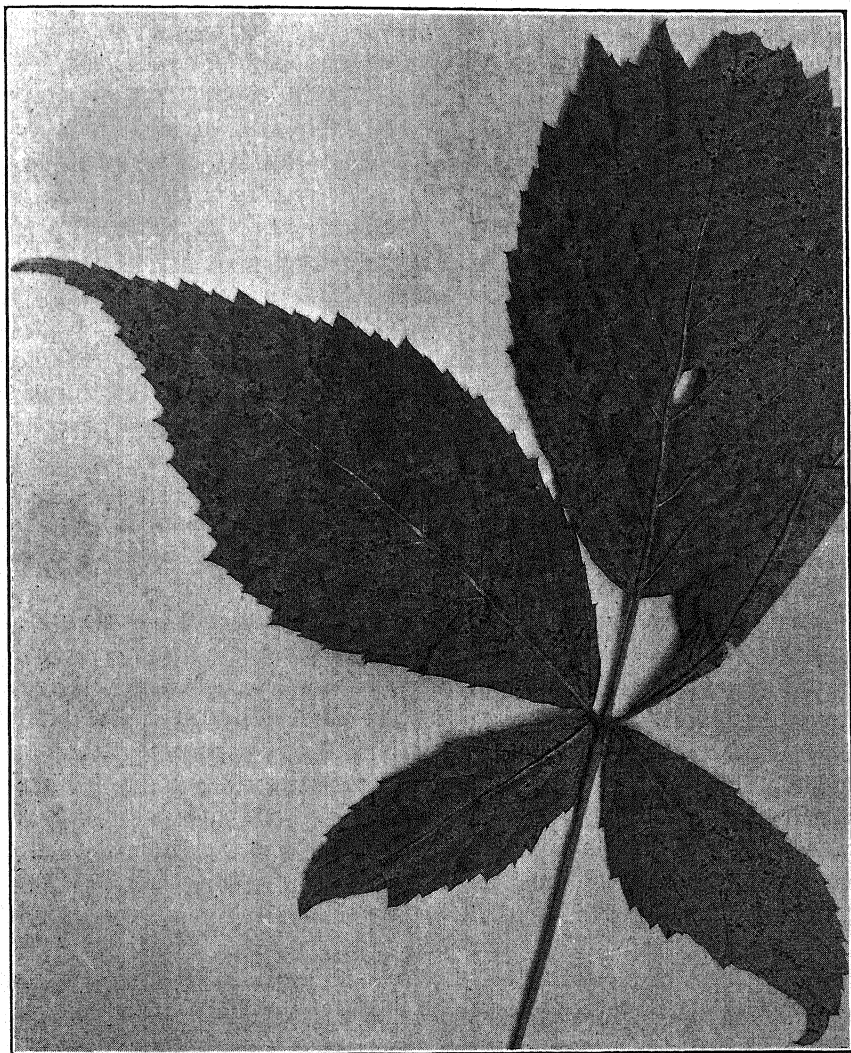


FIG. 5. Telial stage of *Gymnoconia interstitialis* on blackberry, showing localized infections.

In nature, wind-borne spores would fall in greater numbers on the upper surface than on the lower; and, while some infections would no doubt be from spores on the lower surface, the majority undoubtedly took place on the upper surface. In *Diplocarpon rosae* both Aronescu (5) and Dodge (23) have found that upper-surface inoculations gave rise to acervuli on the upper, while lower-surface inoculations produced fruiting bodies also on the lower surface. Gymnoconia, however, bears teliospores on the lower surface irrespective of the point of infection.

DISCUSSION

Tulasne (35) was the first to study the germination of aeciospores. The spores of 9 species were sown on water and, while all formed germ tubes, 2 species, *Aecidium violae* and *Roestelia cancellata* (*Aecidium cancellatum*), produced oval or irregular vesicles at the tips. All the contents moved in and a second germ tube was formed. Similar vesicles were formed on the urediospore germ tubes of *Cronartium asclepiadum*. These were formed either at the end of long germ tubes or close to the spore.

Sappin-Trouffy (31) in 1896 found vesicles on the aeciospore germ tubes of *Uromyces erythronii, Puccinia rubigo-vera, Roestelia lacerata, Peridermium pini,* and also on the urediospore germ tubes of *Puccinia graminis, P. polygonii, Phragmidium rubi* and *Cronartium flaccidum*. According to Sappin-Trouffy, this last species germinated like Tulasne's *Cronartium asclepiadum*. The vesicles described by both Tulasne and Sappin-Trouffy greatly resemble the appressoria of Gymnoconia. The entire contents passed into the tip and Sappin-Trouffy also showed them to be regularly 4-nucleate. According to Sappin-Trouffy, they were never found on the host, and he believed that they were produced only when the germ tube grew out from a submerged spore. A somewhat similar situation has been described in *Gymnosporangium transformans* by Dodge (19). He found that when aeciospores were germinated on water a smaller pouch was formed near the tip of the germ tube. The 2 nuclei moved in and divided once, so that this body contained 4 nuclei. Dodge drew attention to the similarity between these structures and those described by Sappin-Trouffy. These terminal vesicles, found by the above investigators in 7 different species of rusts, probably represent appressoria; had they been formed on leaves, penetration would undoubtedly have taken place. Similar structures on the germ tubes of urediospores suggest that these also are appressoria.

Clinton (16) has stated that in *Gymnoconia interstitialis* infection takes place through the stomata, but the entrance of the germ tubes was not observed. Aeciospores were sown on leaves and a month later teliospores were formed. Details of penetration and the establishment of infection were not given and apparently Clinton assumed that, since the inocula-

tion was successful, the germ tubes had entered through the stomata. Clinton and McCormick (17) produced teliospores in Petri-dish cultures by sowing aeciospores on the lower surface, which bore stomata. Actual entrance of the germ tubes, however, was not observed.

The presence of accessory dorsal stomata on the leaves bearing the haploid systemic mycelium suggested to Dodge (21) that this might account for the presence of teliospores earlier on infected leaves than on healthy leaves. To obtain experimental evidence, aeciospores were dusted on leaves and 2 days later were placed in Flemming's fixative. After fixation the chlorophyll was removed, and the material examined *in toto*. Dodge observed that "the end . . . broadens out and sends an infection tube through the opening." The observation of these details would have been very difficult had not the material been adequately cleared following fixation. The end of the germ tube was observed to "broaden out" and from this broadened tip an infection tube was sent out. It is the writer's belief that this expanded terminal portion represents the appressorium and this is strengthened by the fact that an infection tube develops from it. Were the appressorium to lie directly over the opening, it would certainly seem that the infection hypha were entering through the stoma. As shown in plate I, 9, the infection tube or penetration hypha may enter the guard cells just as readily as any other part of the epidermis.

The production of teliospores earlier on infected leaves may be open to another interpretation. From inoculation experiments made on partially infected leaves (Table 2), it is clear that the infected area with its many accessory stomata possesses no advantage whatsoever over the adjacent healthy stoma-free areas. Nor does "freedom from tomentum" explain the ease of infection, since the lower, very tomentose surface of black-raspberry leaves was penetrated just as readily, the germ tubes growing down among the hairs and forming appressoria on the epidermis (May 14, Table 2). From earlier work (20) and from collections in nature, it is known that teliospores are formed first on the older basal leaves. Since Gymnoconia matures in the spring, healthy leaves—even the basal ones—are still young, while the infected leaves are more or less exhausted by intensive sporulation. They may be said to be "physiologically older" than the neighboring healthy leaves and, accordingly, readily infected and thus would bear teliospores much earlier.

Even when the aeciospores are germinated under different conditions, penetration always takes place by means of an appressorium. Absence of light does not retard penetration in any way. The only factor of importance is temperature. If the temperature rises the percentage of germination drops and various irregularities appear. In the inoculation experiments whenever the temperature in the laboratory was high the percentage of germination and of penetration was low. For instance, in the series of

May 10 (Table 2), 3 Petri dishes were used. In the first 2 the percentage of germination was low, while in the third there was no germination. As both Kunkel (27) and Clinton (16) have pointed out, temperature does not determine how the spores will germinate. In one or two cases long germ tubes are formed on the leaves without any appressoria. Since appressoria usually result through contact stimulus, it may be that this factor was lacking. Hasselbring (26) found that spores of *Gloeosporium fructigenum,* sown on convex drops of water, formed appressoria where they came in contact with the slide, while those that floated on the surface formed long germ tubes but no appressoria.

Penetration rarely occurs unless appressoria are formed. The aeciospore in (Pl. II, 14) is the only exception found. Bolley and Pritchard (13) claim that germ tubes of the urediospores of *Puccinia rubigovera* sometimes penetrated the cuticle without any appressorium. In corn rust, Weber (38) reported that they may or may not be formed. Boyle (14), Blackman and Welsford (12), Bensaude and Keitt (11), working on the other fungi, found little or no formation of appressoria. As Aronescu (5) has pointed out, these cases might indicate that it suffices to have contact between fungus and host.

Penetration of the cuticle apparently obtains in a manner similar to that resulting from germinating basidiospore infection as described by de Bary (10) and Waterhouse (37). A well-defined penetration peg is formed below the appressorium. This is very short and the penetration hypha thickens at a point very close to the cell wall. The literature on cuticular and cell-wall penetration has been adequately reviewed by Dey (18), Rice (29), Arthur (6) and recently by Aronescu (5), and need not be considered here.

An interesting feature of infection is the amount of mycelium developed in the leaf tissues before haustoria are formed. The first haustoria were found on the fifth day, by which time considerable mycelium had been produced. In a globoid spore of 20×25 μ diameter, the volume of cell contents is very large and thus capable of supporting a certain amount of mycelial development. On agar, germ tubes have been observed measuring over 700 μ long. It is to be expected, therefore, that that amount of mycelium could be produced before haustoria are necessary. By the time the first haustorium has been formed, however, the amount of mycelium is considerably greater. Some nourishment must have been obtained directly from the cells of the leaf by the hyphae as they grew through the intercellular spaces. Rice (29) found that corn rust (*Puccinia sorghi*) produced abundant mycelium before the first haustorium was formed, about the fifth day after inoculation. These results agree very closely with those of *Gymnoconia interstitialis.* In *Acanthostigma parasitica,* the causal agent of thread blight of tea, Butler (15) found that the fungus forms web-like films of hyphae over the lower surface of the leaf. There are no haustoria

or penetrating organs and the wefts of mycelium may be readily stripped from the leaf. From this he contends that the food may pass to the fungus through the unbroken walls of the cell.

Since the short-cycle rust becomes systemic following its attack of the young shoots, several inoculations with aeciospores of the long-cycle rust were attempted. If the diploid mycelium could enter the shoot it would be able to spread up the cane into the young leaves and down into the roots. Theoretically, this is possible, since stomata are unnecessary. The results of the inoculations, however, were negative. Germination occurred readily and appressoria were formed but no penetration took place. Inoculations on seedlings gave teliospores only on the older basal leaves, none being found on the very young leaves. Maturity of the tissues is apparently an important factor in the establishment of infection. Similar results were noted by Sheldon (32) in *Puccinia asparagi*. Seedlings of *Alium cepa* were completely immune, but when about 2 months old, were favorable for the development of aecia.

Although it generally is accepted that the entrance of aeciospore germ tubes is through the stomata (Arthur 6), an examination of the investigations made on this point reveals that comparatively few species have actually been studied. De Bary (9) made germination tests of both aeciospores and urediospores from 32 species. He was the first to observe their behavior when sown on the leaves of their hosts. In all cases the germ tubes were found to grow to the stomata and enter through the opening. Unfortunately, the number of species investigated in this fashion is not given, but, in a table recording the results of many cross-inoculations, 5 cases of aeciospore penetration are noted. In *Aecidium cyparissiae, A. tragopogonis, A. taraxaci, A. asperifolii* and *Uromyces appendiculatus* the germ tubes entered the stomata of not only their own hosts but also of other unrelated hosts. In these latter cases de Bary has pointed out that no infection took place. He came to the conclusion that in the rusts there are two methods of penetration. In one case the germ tube penetrates the cuticle. This takes place with the basidiospores developed from the promycelium. In the other case the germ tubes penetrate the host by means of the stomatal opening. This, de Bary concluded, is the method of aeciospore and urediospore penetration. Gibson (25) studied the behavior of 6 species on both their own and other hosts and found the aeciospore germ tubes always entered through the stomata. She concluded that this method of penetration was normal. Stomatal entrance has been observed by Clinton and McCormick (17) (*Cronartium ribicola*), and recently by Zeller and Lund (39) (*Phragmidium rubi-idaei*), and Ashworth (7) (*Coleosporium tussilaginis*). In these cases aeciospore infection through the stomata seems to be well established.

The emphasis of much of the recent work has been rather upon the

entrance of the urediospore germ tube. The researches of Ward (36), Evans (24), Stakman (34), Ruttle and Fraser (30), Allen (1, 2, 3, 4), Rice (29), and others have seemingly established the fact that infection from urediospores takes place through the stomatal openings. Since de Bary (9) and Gibson (25) concluded that the aeciospores and urediospores behave in the same way on their hosts it has been assumed that *all* aeciospores penetrate in the same way. When Clinton and McCormick (17) made their extensive series of Petri-dish inoculations they sowed aeciospores on those surfaces known to bear stomata. The successful infections obtained in 9 species were assumed to be the result of stomatal penetration. In order to make general statements as to the behavior of the aeciospore germ tubes, it is necessary to know the behavior of a large number of species. Until such data are available, it cannot be assumed that all species behave similarly. Our present knowledge of aeciospore germ-tube penetration is insufficient to justify the assumption that stomatal penetration is normal. Moreover, the discovery of appressoria on the germ tubes of the aeciospores of *Gymnoconia interstitialis* and their function in penetration of the cuticle, as well as the number of appressorium-like bodies described by many authors, suggests that stomata are not always necessary for successful infection from aeciospores.

SUMMARY

The results of a series of inoculations with the aeciospores of the long-cycle rust, *Gymnoconia interstitialis,* show that infection takes place by means of appressoria that are formed at the end of the germ tubes. The entire contents of the aeciospore enter and the 2 nuclei divide once, so that the mature appressorium is 4-nucleate. A well-marked penetration peg passes through the cuticle and cell wall and a short penetration hypha is formed in the epidermal cell. The lower wall of the host cell is penetrated and the hypha enters the intercellular space just below. The mycelium spreads rapidly in the leaf tissue and haustoria may be observed after 5 days. Teliospores were formed from 21 days on. Penetration takes place equally well through the upper stoma-free surface and on the lower surface of leaves of either the blackberry, black raspberry, or dewberry. Leaves, already infected with the haploid stage, were penetrated in the same manner, the 2 mycelia not being antagonistic. Inoculations on partially infected leaves show that infection occurs just as readily on the healthy stoma-free area as on the infected area, which bears accessory stomata. Penetration may thus take place at any point on the leaf irrespective of the presence or absence of stomata.

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EXPLANATION OF PLATES

All figures drawn with camera lucida from "in toto" preparations and shading is an attempt to represent the differences in level as one focuses downward. Darker parts are deeper in the leaf tissue.

Plate I. 1–2. Young appressoria at end of short germ tube. 3. Mature appressorium with four nuclei. 4. Penetration on midrib showing penetration peg (c) and young penetration hypha (d). 5. Typical penetration hypha (d) in epidermis (24 hrs.). 6. Young intercellular hypha (e) from penetration hypha (d). 7. Penetration on infected black-raspberry leaf. Hypha at f pushing downward in palisade. Note the accessory stomata. 8. Same as fig. 7. Penetration hypha (d) has branched in epidermis. A haustorium (g) from the systemic mycelium is in the same cell. 9. Penetration hypha (d) in guard cell. Intercellular mycelium (e) well advanced in mesophyll. a, aeciospore; b, appressorium; c, penetration peg; d, penetration hypha in epidermis; e, mycelium in intercellular spaces beneath epidermis; f, mycelium in palisade. ×750.

Plate II. 10. Penetration at base of pycnium (p) on infected black raspberry (24 hrs.). 11–12. Typical infections on lower epidermis (48 hrs.). 13. Penetration at leaf edge showing binucleate condition. Host cells omitted from drawing. 14. Penetration without an appressorium. Penetration peg (c) formed at germ pore (11 days). The explanation of the letters is given in legend for Plate I. ×750.

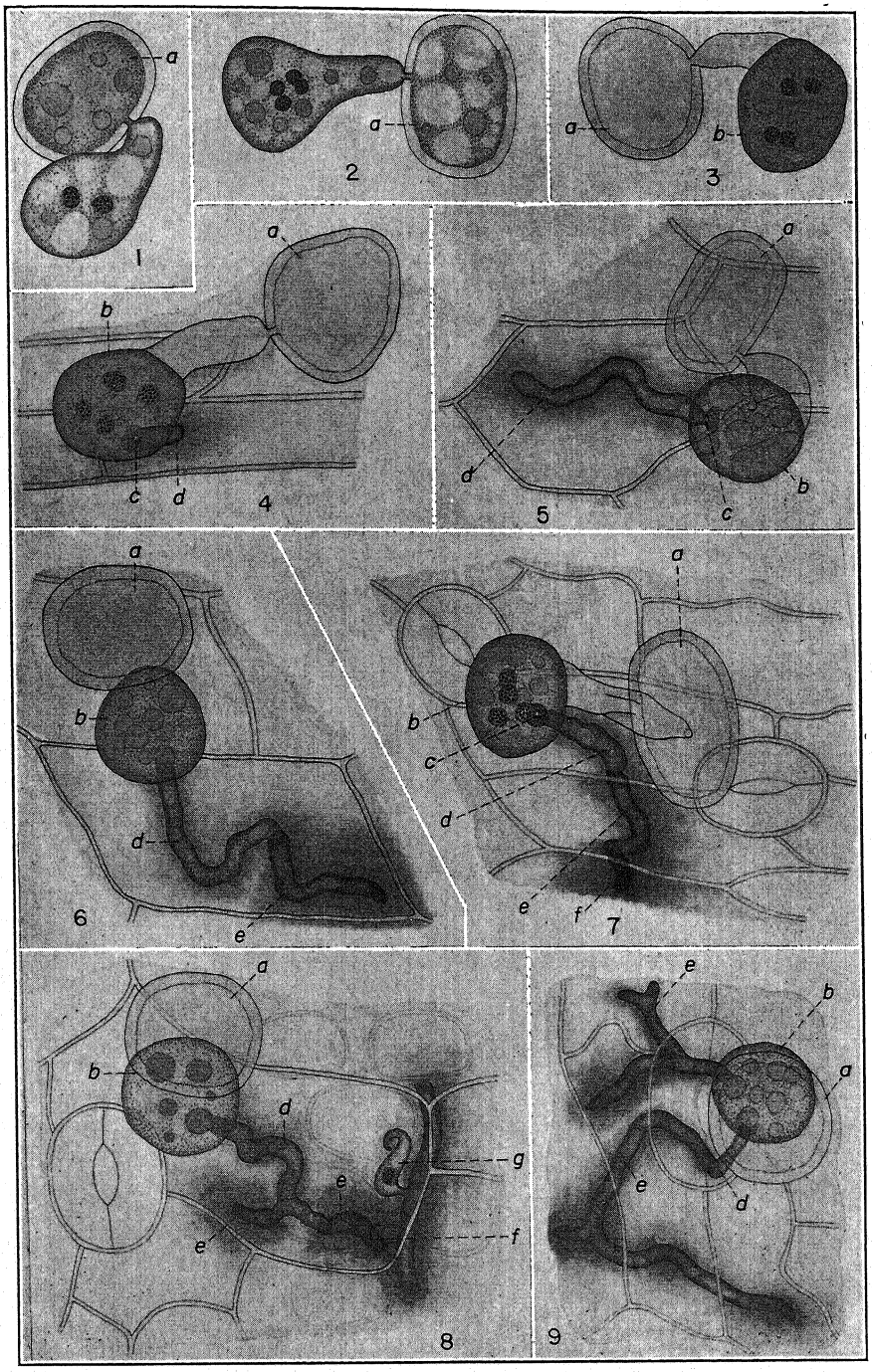


PLATE I

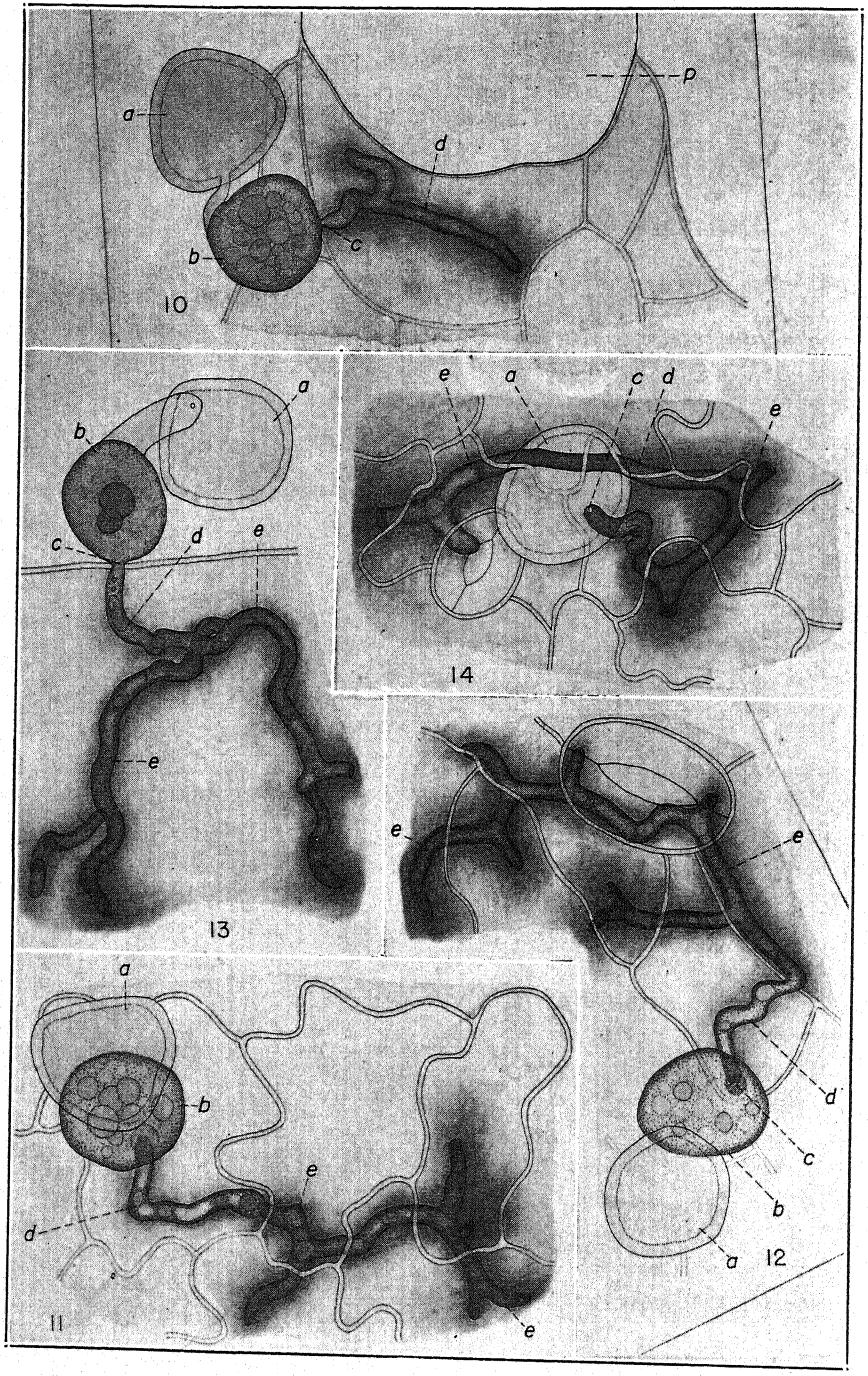


PLATE II

CHEMICAL STUDIES ON THE VIRUS OF TOBACCO MOSAIC
III. RATES OF INACTIVATION AT DIFFERENT HYDROGEN-ION CONCENTRATIONS

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INTRODUCTION

A complete study of the chemical treatment of preparations of tobacco-mosaic virus necessitates knowledge of the range of hydrogen-ion concentration over which the virus is stable in order to distinguish inactivation due to excessive acidity or alkalinity from that due to other causes. In the concentration and purification of virus, the success of many precipitation, adsorption, and elution procedures, and of many chemical reactions, is dependent on the ability of virus to withstand certain hydrogen-ion concentrations. However, little is known of the effects of hydrogen-ion concentration on virus, there being no definite study on this subject and the few incidental observations being, in some instances, contradictory. One of the first reports in this connection was that of Allard (1) who, while engaged in a study of the effect of many chemicals on tobacco-mosaic virus, found that 1 per cent nitric or hydrochloric acid inactivated virus, but that 0.5 per cent acid left the virus infectious. Allard also reported that 0.1 per cent sodium hydroxide inactivated the virus, whereas it was not inactivated by 0.025 per cent sodium hydroxide. He did not measure the hydrogen-ion concentration of the solutions, and because of the differences in the buffering ability of different samples of plant juices it is impossible to translate his results to the pH scale. Nevertheless, Allard's findings indicate that excessive acidity or alkalinity inactivates virus and that alkaline reactions are probably more harmful than acid reactions. Brewer, Kraybill, Samson, and Gardner (3) reported that they found virus active at hydrogen-ion concentrations as high as pH 2.46, and that it was inactivated at pH 8.4 or pH 8.5, but, most strikingly, was reactivated when the concentration was brought to pH 7.74 or pH 5.33. This reactivation of virus has been neither disproved nor confirmed, although Vinson and Petre (16) found that complete inactivation did not occur in 24 hours at pH 10.5 and that the addition of acid did not restore the activity of solutions inactivated above pH 9.2. Fukushi (6) reported the virus to be active at pH 2 and pH 8.4, and to be most infectious between pH 4 and

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pH 7. Lojkin and Vinson (11) reported inactivation of virus at pH 3.3, but Vinson and Petre (16) found that other samples of virus retained their infectivity at pH 1.8.

The differences in the results obtained by different investigators are probably due to many factors, such as different methods of inoculation of virus, different test plants, different hydrogen-ion concentrations at the time of inoculation, different periods of time of exposure to the various hydrogen-ion concentrations, and to errors arising during the measurement of the hydrogen-ion concentration. Therefore, data available at present indicate only that virus is inactivated by excessive acidity or alkalinity, there being no complete data available regarding definite hydrogen-ion concentrations that either do or do not inactivate virus, and no data concerning the rates of inactivation of virus at different hydrogen-ion concentrations. As a preliminary to a chemical study on the virus of tobacco mosaic, it was necessary to secure these data. The results should be useful not only to chemists but to others working in the virus field, and they may serve to give an additional insight into the nature of viruses. The present paper records the results of a study of the rates of inactivation of tobacco-mosaic virus at different hydrogen-ion concentrations.

MATERIALS AND METHODS

The virus used in the experiments here reported consisted of diluted untreated infectious juice and a purified virus preparation. The untreated infectious juice was obtained from plants of mosaic-diseased *Nicotiana tabacum* L. variety Turkish. The plants were cut and kept in a freezing room for a few days and then allowed to thaw just before pressing. The juice was filtered through 2 layers of bandage gauze and then diluted with 2 volumes of distilled water. The hydrogen-ion concentration of the juice was pH 5.4 before dilution and pH 5.7 after dilution. The purified virus was prepared from undiluted infectious juice by precipitation with lead acetate and elution with potassium phosphate, according to a modification of the method described by Vinson and Petre (16). This preparation was diluted with 9 parts of 0.1 M phosphate at pH 6.8. Then 1 per cent by weight of charcoal (Suchar 00) was added while stirring and the mixture was filtered through hardened filter paper 4 times. This purified preparation was colorless, water-clear, and somewhat more infectious than the diluted untreated infectious juice.

All determinations of hydrogen-ion concentration were made by means of a MacInnes type glass electrode (12) in order to eliminate errors due to foreign substances in the virus preparations. Unlike the hydrogen electrode, quinhydrone electrode, and color indicators, the glass electrode is unaffected by foreign substances such as electrode poisons, salts, reduc-

ing and oxidizing agents. The glass electrode is, therefore, especially suitable for the determination of hydrogen-ion concentrations of plant extracts. The apparatus used is similar to that described by Hill (7) and is capable of an accuracy of 0.001 pH unit.

In general, the experimental procedure used in the 3 sets of experiments to be reported consisted in adjusting separate portions of 250 cc. or more of the virus preparations to hydrogen-ion concentrations of from pH 0.5 to pH 12, in either 0.5 pH or 1.0 pH gradations, by the addition of acid or alkali. Immediate tests for virus infectivity at the various hydrogen-ion concentrations were made by the half-leaf method of inoculation, as described previously (9, 14, 15). After standing for various periods of time, portions of each of the preparations at the various hydrogen-ion concentrations were removed and adjusted to about pH 6 and tested against a control for virus infectivity. These tests were made by comparing the average number of lesions per half-leaf obtained on 5 or more half-leaves of *Nicotiana glutinosa* L. or 12 or more half-leaves of *Phaseolus vulgaris* L. variety Early Golden Cluster on inoculation with the various preparations, with the average number of lesions obtained on the other halves of the same leaves on inoculation with the standard or control virus preparation. In 2 experiments the virus preparations were kept frozen except during the tests for virus infectivity, but in a third experiment the preparations were kept at room temperature. Toluol was added to prevent bacterial action during the latter experiment.

In order to avoid using large volumes of acid or alkali and thus introducing an error due to dilution of the virus, solutions of 2.0 N HCl and NaOH were usually used to adjust the hydrogen-ion concentration. In certain instances concentrated HCl or a saturated solution of NaOH were used. The volume of the virus preparation was in no instance changed more than 7 per cent by the addition of acid or alkali. The error introduced by dilution of the virus to this extent is very small and may be neglected. In order to eliminate errors due to inactivation of virus by local excessive concentration of acid or alkali, the HCl or NaOH was added dropwise while stirring the virus preparation vigorously with a mechanical stirrer.

EXPERIMENTAL

Rates of Inactivation of Virus in Diluted Untreated Juice Prepared from Frozen Plants

Plants of *Nicotiana tabacum* variety Turkish were infected with typical tobacco-mosaic virus, when about 3 or 4 inches high, and grown in a greenhouse. When they had reached the stage of maturity indicated by the beginning of flower-bud formation, they were cut and placed in

a room held at −14° C. for a few days. The frozen material was allowed to thaw and the juice pressed out and filtered through 2 layers of bandage gauze. Two liters of filtered juice were diluted with 4 liters of distilled water. Twelve portions of 300 cc. or more of this diluted untreated infectious juice were then taken to hydrogen-ion concentrations of from pH 1 to pH 12 in gradations of 1 pH, by the addition of 2 N NaOH or HCl. In one instance, concentrated HCl was used in order to reduce the volume of the added acid. The amount of diluted untreated infectious juice used for each hydrogen-ion concentration is listed in the first column of table 1. The amount of acid or alkali needed to adjust each preparation is given in the second column, and the hydrogen-ion concentration of the adjusted preparation is given in the third column. It may be seen that the addition of acid or alkali did not change the volume of the preparations by more than 5 per cent, and that the hydrogen-ion concentrations were adjusted to within 0.05 pH of the desired values. Immediately after adjusting each preparation to the desired hydrogen-ion concentration, a small portion was removed and tested, at that hydrogen-ion concentration, for virus infectivity by the whole-leaf method on *Phaseolus vulgaris*. Additional 50 cc. portions of each of the preparations were removed and, after standing for 1 hour at 20° C., were readjusted to pH 6.0 ± 0.2 and then tested for virus infectivity against the control, using the half-leaf method of inoculation on *P. vulgaris* and *Nicotiana glutinosa*. The amount of 2 N NaOH or HCl necessary to adjust 50 cc. of each of the preparations to pH 6.0 ± 0.2 is given in the last column of table 1. On addition of acid or alkali, a precipitate forms when the hydrogen-ion concentration becomes too high or too low. This precipitate forms on the alkaline side at about pH 8 and becomes greater as the hydrogen-ion concentration is further reduced to pH 12, the largest amount of precipitate occurring at pH 12. Since conditions at pH 8 to pH 12 are unfavorable for adsorption of virus, removal of this precipitate by filtration or centrifugation could be used as a clarification process without great loss of virus. In the case of the precipitate that forms on the acid side, when the hydrogen-ion concentration is increased to about pH 2, conditions favor adsorption of virus, hence removal of precipitate would also tend to remove virus. Therefore, the precipitates were allowed to remain and the virus preparations were well stirred in order to distribute the precipitates evenly before removal of samples for test purposes.

Immediately after adjustment to the various hydrogen-ion concentrations, the main portion of each of the preparations and an untreated control were placed in a room held at −14° C. After standing for 2 days, the 12 preparations and the control were removed, allowed to thaw, and the hydrogen-ion concentration of each was determined. The results, listed

TABLE 1.—*Record of amount of acid or alkali required to adjust preparations, of hydrogen-ion concentrations at the end of various time periods, and of amount of acid or alkali required to readjust preparations*

Amount of diluted untreated juice used	Amount of acid or alkali used	pH of adjusted preparation	pH after 2 days. Before readjustment	pH after 9 days. Before readjustment	pH after 36 days. Before readjustment	pH after 4 months. Before readjustment	pH after 12 months. Before readjustment	Amount of 2N acid or alkali used to adjust 50 cc. of each preparation to pH 6.0 ± 0.2
400 cc.	5 cc. conc. HCl + 15 cc. 2N HCl	1.00	1.06	1.05	1.09	0.93	.....	4.02 cc.
550 cc.	15 cc. 2N HCl	2.01	2.05	2.07	2.02	2.06	.....	1.47 cc.
300 cc.	4 cc. 2N HCl	3.05	3.11	3.02	2.94	3.00	3.03	0.60 cc.
300 cc.	2.2 cc. 2N HCl	4.00	4.12	3.98	3.89	3.98	4.00	0.40 cc.
300 cc.	0.44 cc. 2N HCl	5.04	5.12	5.00	4.88	5.06	5.04	0.08 cc.
300 cc.	0.12 cc. 2N NaOH	6.03	6.09	5.94	5.90	6.22	6.04	.....
300 cc.	0.54 cc. 2N NaOH	7.05	7.20	6.89	6.89	7.11	7.05	0.05 cc.
550 cc.	1.5 cc. 2N NaOH	8.00	8.00	7.75	7.74	7.97	7.85	0.10 cc.
550 cc.	2.08 cc. 2N NaOH	9.01	8.88	8.60	8.67	8.88	8.50	0.20 cc.
300 cc.	2.0 cc. 2N NaOH	9.97	9.70	9.66	9.37	9.80	9.75	0.59 cc.
300 cc.	3.5 cc. 2N NaOH	11.05	10.98	10.77	10.21	11.15	10.81	0.69 cc.
300 cc.	15.0 cc. 2N NaOH	12.00	12.07	12.03	11.46	11.62	11.35	2.06 cc.

in the 4th column of table 1, indicate that some of the preparations were more than 0.05 pH from the desired hydrogen-ion concentrations. These preparations were, therefore, readjusted to within 0.05 pH of the desired values. Additional 50 c.. portions of each were removed, adjusted to pH 6.0 $\pm$ 0.2, and tested on *Phaseolus vulgaris* and *Nicotiana glutinosa* against the control. The main portion of each preparation was again placed in a room held at $-14°$ C. At the end of 9 days, 36 days, 4 and 12 months, the above-described procedure was repeated. The hydrogen-ion concentrations of the various preparations before readjustment and at the end of the various time periods are given in columns 5 to 8, inclusive, of table 1. It may be seen that the preparations at pH 1 to pH 9 remained fairly close to the desired values, but that the preparations at pH 10, pH 11, and pH 12 became changed by as much as 0.79 pH. This marked change occurred during the longer time periods when all or most of the virus had already been inactivated and did not, therefore, introduce serious errors.

The result of the tests for virus infectivity are given in table 2. The actual number of lesions per half-leaf obtained with each preparation at the end of each period of time is given for every preparation. An averaged number which is the quotient of the number of lesions obtained per half-leaf on inoculation with the various preparations, divided by the number of lesions obtained on the other halves of the same leaves on inoculation with the control or untreated virus preparation, is also given. When this averaged number approximates 100, it indicates that the preparation produced about as many lesions as did the control preparation, and that little or no inactivation of virus occurred. As this number decreases, it indicates that a decreasing amount of virus is present. When no lesions are produced on inoculation of *Nicotiana glutinosa* or *Phaseolus vulgaris,* the quotient is zero and inactivation is complete. Since the control was the same in all instances, each number is related to all other numbers. This makes possible a comparison of the amount of virus, as evidenced by the number of lesions produced on inoculation, remaining in each of the preparations at the end of the different time periods. It should be emphasized, however, that as others (2, 4, 5, 8, 9, 13, 14) have indicated, the relation of number of lesions to virus concentration does not approach a direct proportionality, except possibly at high dilutions. An idea of the actual relative amount of virus present can be obtained only by comparison of the number of lesions produced by any given sample with the numbers of lesions produced by dilutions of the control virus sample, the relative concentrations of which are known. It is also necessary that the virus preparation be sufficiently diluted so that on further dilution fewer lesions are produced on inoculation. The original virus preparations used in all experiments described in this paper were appropriately diluted. If, therefore, under

TABLE 2.—*Tests for virus infectivity of diluted untreated infectious juice at different hydrogen-ion concentrations immediately after adjustment and at pH 6, after standing at different hydrogen-ion concentrations at −14° C. for periods of time up to 1 year*

Preparations maintained at pH	Test plant	Preparations tested at different pH's immediately after adjustment	Preparations tested at pH 6 after being held 1 hour at different pH's		Preparations tested at pH 6 after being held 2 days at different pH's		Preparations tested at pH 6 after being held 9 days at different pH's		Preparations tested at pH 6 after being held 36 days at different pH's		Preparations tested at pH 6 after being held 4 months at different pH's		Preparations tested at pH 6 after being held 12 months at different pH's	
		Actual	Averaged[a]	Actual[b]	Averaged	Actual	Averaged	Actual	Averaged	Actual	Averaged	Actual	Averaged	Actual
1	N. glutinosa		37.1	30.8	2.2	2.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	P. vulgaris	0.12	15.6	14.3	0.1	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2	N. glutinosa		82.3	58.3	37.6	35.5	2.8	2.0	0.0	0.0	0.0	0.0	0.0	0.0
	P. vulgaris	4.7	74.0	69.8	27.1	29.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3	N. glutinosa		106.0	87.5	97.2	78.5	80.5	79.4	95.0	98.4	74.0	24.8	79.5	78.6
	P. vulgaris	6.3	69.4	58.5	87.5	94.7	165.0	89.0	154.0	71.7	69.6	19.9	88.5	9.2
4	N. glutinosa		114.1	55.8	85.8	67.4	102.8	125.0	124.6	98.0	64.5	39.7	75.0	70.7
	P. vulgaris	23.2	88.0	70.1	91.5	147.0	145.0	71.7	178.0	132.0	72.1	34.2	98.0	8.3
5	N. glutinosa		98.1	76.8	140.5	112.0	104.0	98.8	72.1	86.9	94.5	56.0	96.5	86.4
	P. vulgaris	55.6	88.3	53.2	90.0	116.2	146.0	91.5	125.7	103.7	69.6	37.6	82.5	13.4
6	N. glutinosa		100.8	43.0	132.5	79.4	116.1	94.6	109.8	79.5	89.0	63.0	90.0	96.6
	P. vulgaris	74.0	91.2	82.1	80.0	121.1	127.7	87.0	166.0	85.5	74.9	27.6	73.4	7.6
7	N. glutinosa		92.3	79.2	105.7	49.7	99.8	106.5	98.6	86.9	128.0	94.0	86.0	95.7
	P. vulgaris	71.5	99.8	55.5	79.5	105.0	110.0	71.0	162.2	107.0	95.5	34.5	86.0	9.7
8	N. glutinosa		112.1	83.2	112.0	64.6	80.0	55.6	112.2	74.6	91.0	66.5	94.5	73.4
	P. vulgaris	98.9	79.0	62.5	69.5	101.0	143.7	96.5	156.0	68.5	71.5	13.7	43.0	1.8
9	N. glutinosa		94.4	70.9	88.9	74.8	91.5	60.0	60.3	63.7	48.4	31.3	37.9	19.9
	P. vulgaris	92.5	72.9	74.1	65.8	71.6	98.5	61.3	71.7	39.1	40.2	7.5	22.1	0.8
10	N. glutinosa		79.2	70.0	49.2	43.0	26.4	18.0	9.7	11.0	3.3	2.8	1.2	1.9
	P. vulgaris	59.8	68.5	36.4	22.3	29.6	11.0	7.2	0.8	0.5	0.0	0.0	0.0	0.0
11	N. glutinosa		0.4	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	P. vulgaris	11.4	0.2	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
12	N. glutinosa		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	P. vulgaris	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

[a] Numbers represent the quotient of the average number of lesions per half-leaf obtained on 5 or more half-leaves of *Nicotiana glutinosa* or 12 or more half-leaves of *Phaseolus vulgaris* on inoculation with the designated preparation, divided by the average number of lesions obtained on the other halves of the same leaves on inoculation with the control preparation.

[b] Numbers represent the average of the actual number of lesions per half-leaf obtained on 5 or more half-leaves of *N. glutinosa* or 12 or more half-leaves of *P. vulgaris* on inoculation with the designated preparation.

the same conditions the sample produces about the same number of lesions as the control diluted 1 to 10, it may be assumed that about 90 per cent of the virus has been inactivated. If the sample produces about the same number of lesions as the control diluted 1 to 100, then about 99 per cent of the virus has been inactivated. Tests of dilutions of the control preparations by the half-leaf method are given in table 5 in order to permit estimation of the actual amount of virus present in the various preparations. An idea of the rate of inactivation of virus at different hydrogen-ion concentrations may thus be obtained.

The results given in table 2 show that tobacco-mosaic virus is inactivated immediately at pH 12, very rapidly at pH 11 and at pH 1, and fairly rapidly at pH 10 and pH 2. The results also indicate that the virus is quite stable at pH 3 to pH 8 inclusive, and fairly stable at pH 9, for periods as long as 1 year. The rate of inactivation of tobacco-mosaic virus in diluted untreated juice prepared from frozen plants is, therefore, extremely slow at hydrogen-ion concentrations between pH 3 and pH 8, and very rapid at hydrogen-ion concentrations more acid than pH 3 and more alkaline than pH 9. Virus preparations completely inactivated at pH 1, pH 2, pH 11, and pH 12, and then adjusted to pH 6 were tested for virus infectivity after standing for periods of time as long as 1 year, and in no case was virus demonstrated. This is an indication that virus inactivated at unfavorable hydrogen-ion concentrations, such as pH 1, pH 2, pH 11, and pH 12, does not become reactivated on long standing at pH 6, a hydrogen-ion concentration that may be considered favorable to the virus.

Rates of Inactivation of Virus in Juice from Macerated Fresh Leaves in Critical Regions near pH 2 and pH 10

In the experiment just described, no tests were included for periods between 1 hour and 48 hours and only 6 preparations were at hydrogen-ion concentrations more acid than pH 3.5 and more alkaline than pH 9.5, the two regions in which the rate of inactivation becomes very rapid. It seemed desirable to make tests at shorter time intervals and at a greater number of different hydrogen-ion concentrations in the regions near pH 2 and pH 10. Since some investigators work at room temperature with virus extracts prepared from freshly macerated tissues, it seemed desirable to make tests on such extracts held at room temperature. An experiment was made, therefore, for the purpose of determining the rates of inactivation at room temperature (20° C.), in the regions around pH 2 and pH 10, of virus in juice prepared from fresh tissue. Leaves from plants of *Nicotiana tabacum* variety Turkish, diseased with tobacco mosaic, were removed, put through a meat grinder, and the juice pressed out.

This juice, which was at pH 5.7, was centrifuged, diluted with 2 volumes of distilled water, and saturated with toluol. After standing overnight at room temperature, the hydrogen-ion concentration of the preparation was pH 7.1. Thirteen 300 cc. portions of this preparation were held at room temperature (20° C.) for 192 hours, at hydrogen-ion concentrations of from pH 0.5 to pH 3.5 and from pH 9.5 to pH 12 in gradations of 0.5 pH. The adjustment of the preparations to the desired hydrogen-ion concentrations and the readjustment of portions of each to, in this experiment, pH 6.0 ± 0.4 was done in exactly the same manner as that described for the first experiment. The amounts of acid and alkali required were comparable to the amounts used in the first experiment. The precipitates which formed in these preparations were well mixed before removal of samples for test purposes.

Tests for virus infectivity were made by the half-leaf method on each preparation immediately after adjustment to the different hydrogen-ion concentrations, at these hydrogen-ion concentrations, and also after being readjusted to pH 6.0 ± 0.4. Tests were also made at pH 6.0 ± 0.4 on preparations that had stood at room temperature at the various hydrogen-ion concentrations for time periods of 1, 5, 20, 48, 96, and 192 hours. In this experiment the different preparations were not readjusted to the initial hydrogen-ion concentrations at the end of the various time periods, since the rate of hydrolysis at pH 9.5 to pH 12 at room temperature was found to be so great as to cause the preparations to require readjustment every few hours in order to maintain fairly constant hydrogen-ion concentrations. It may be seen from table 3 that preparations initially at pH 9.5 to pH 12 became changed by as much as 2.1 pH during the course of 8 days. The results obtained with preparations at pH 9.5 to pH 11 are, therefore, indicative of inactivation in preparations initially adjusted to the given hydrogen-ion concentrations and then allowed to stand without further treatment, and not of preparations held at constant hydrogen-ion concentrations. The results obtained with preparations at all other hydrogen-ion concentrations are, however, indicative of inactivation in preparations held at constant hydrogen-ion concentrations, since, at pH 11.5 and pH 12.0, inactivation of virus was completed before the hydrogen-ion concentration changed appreciably, and at pH 0.5 to pH 3.5 there was but little change in the hydrogen-ion concentration of the preparations during the course of 8 days. The results of the tests for virus infectivity (Table 3) show that the virus is remarkably stable between pH 1.5 and pH 10.0 for periods as long as 20 hours and even somewhat stable at pH 1.0, pH 10.5, and pH 11.0 for the same length of time. It is completely inactivated at pH 12 and almost completely inactivated at pH 0.5 immediately after adjustment to these hydrogen-ion concentrations. At pH

TABLE 3.—*Tests for virus infectivity at different hydrogen-ion concentrations immediately after adjustment and at pH 6, after standing at different hydrog concentrations at 20° C. for periods of time up to 192 hours. Untreated infectious juice prepared from fresh leaves*

pH of preparations at start	Test plant	Preparations tested at different pH's immediately after adjustment		Preparations tested after an immediate readjustment to pH 6		Preparations tested at pH 6 after being held 1 hour at different pH's		Preparations tested at pH 6 after being held 5 hours at different pH's		Preparations tested at pH 6 after being held 20 hours at different pH's		Preparations tested at pH 6 after being held 48 hours at different pH's		Preparations tested at pH 6 after being held 96 hours at different pH's		Preparations tested at pH 6 after being held 192 hours at different pH's		pH of preparations at end of 8 days
		Averaged[a]	Actual[b]	Averaged	Actual	Averaged	Actual	Averaged	Actual	Averaged	Actual	Averaged	Actual	Averaged	Actual	Averaged	Actual	
0.5	N. glutinosa	0.0	0.0	0.1	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.6
	P. vulgaris	0.0	0.0															
1.0	N. glutinosa	4.6	8.8	53.4	104.6	59.5	139.8	61.0	102.6	56.5	85.8	1.3	1.7	0.0	0.0	0.0	0.0	1.1
	P. vulgaris	0.0	0.0									1.0	0.4					
1.5	N. glutinosa	23.1	35.8	85.6	138.2	96.0	232.8	85.0	204.2	82.5	139.0	50.5	86.0	50.4	107.2	56.0	121.0	1.5
	P. vulgaris	1.1	0.3									79.0	50.5					
2.0	N. glutinosa	74.8	137.0	73.5	149.4	88.4	177.4	98.5	241.2	69.6	131.6	90.8	161.8	55.4	95.6	58.0	122.0	2.0
	P. vulgaris	6.8	1.7									140.0	75.5					
2.5	N. glutinosa	87.0	194.4	70.4	184.4	83.2	157.6	83.0	208.0	77.2	106.8	115.0	179.8	84.5	180.0	78.0	127.0	2.5
	P. vulgaris	11.5	3.0									184.0	52.4					
3.0	N. glutinosa	77.7	169.2	65.4	126.4	77.0	191.8	79.4	267.0	90.5	137.8	109.1	206.6	90.0	159.2	81.3	179.6	3.0
	P. vulgaris	10.4	4.2									159.0	75.9					
3.5	N. glutinosa	101.0	160.0	83.8	95.0	90.8	151.8	95.5	158.8	99.0	109.6	100.0	136.6	82.0	148.0	96.5	113.4	3.5
	P. vulgaris	10.6	0.8									435.0	85.0					
4.5	N. glutinosa	86.5	144.0	97.2	166.6	112.7	122.6	102.5	188.4	95.0	139.0	92.5	183.8	63.8	124.6	74.2	118.4	8.5
	P. vulgaris	199.5	59.8									66.5	17.7					
5.0	N. glutinosa	92.5	186.8	94.8	179.8	77.5	136.4	86.0	201.2	91.5	142.6	82.2	166.0	83.0	166.4	71.3	97.4	8.6
	P. vulgaris	485.0	121.0									10.0	0.6					
5.5	N. glutinosa	84.6	209.2	81.4	92.4	67.7	138.4	74.2	159.8	36.4	67.8	34.5	43.4	63.6	110.8	39.2	26.8	8.9
	P. vulgaris	323.0	28.4									10.3	3.4					
6.0	N. glutinosa	56.8	111.6	80.5	189.4	78.0	117.2	76.7	138.6	49.6	97.2	27.6	52.8	50.6	110.0	46.5	60.8	9.2
	P. vulgaris	55.1	8.4									2.4	0.7					
6.5	N. glutinosa	1.7	3.4	35.8	84.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	9.4
	P. vulgaris	0.0	0.0															
7.0	N. glutinosa	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	11.1
	P. vulgaris	0.0	0.0															

[a] Numbers represent the quotient of the average number of lesions per half-leaf obtained on 5 or more half-leaves of *Nicotiana glutinosa* or 12 or more half-leave *seolus vulgaris* on inoculation with the designated preparation, divided by the average number of lesions obtained on the other halves of the same leaves on in on with the control preparation.

[b] Numbers represent the average of the actual number of lesions per half-leaf obtained on 5 or more half-leaves of *N. glutinosa* or 12 or more half-leaves of *aris* on inoculation with the designated preparation.

1.0, standing for between 48 and 96 hours was required for complete inactivation, while at pH 11.5 less than 1 hour was required for complete inactivation. Preparations held at pH 1.5 for as long as 192 hours still gave about 50 per cent as many lesions as the untreated control. It is evident that the virus may be subjected to a very wide range of hydrogen-ion concentrations for fairly long periods of time during chemical manipulation without much loss of activity.

Rates of Inactivation of Virus in Purified Preparations

In order to determine whether the large amount of extraneous matter present in untreated juice has an effect on the rate of inactivation of virus, a third experiment was made using a purified preparation of virus in place of diluted untreated infectious juice. A 350 cc. portion of a virus preparation purified by a modification of the lead acetate process described by Vinson and Petre (16) was diluted to 3.5 liters with 0.1 M phosphate at pH 6.8, and 35 g. of charcoal (Suchar 00) was added. After stirring for 5 minutes, the mixture was filtered through hardened filter paper 4 times. Then 250 cc. portions were taken to different hydrogen-ion concentrations, readjusted to about pH 6.8 after standing for different periods of time at −14° C., and tested on *Phaseolus vulgaris* and *Nicotiana glutinosa* in exactly the same manner as described in the first experiment in which diluted untreated infectious juice was used. Due to the absence of the large amount of extraneous materials capable of acid or alkaline hydrolysis, the purified preparations remained very close to the desired hydrogen-ion concentrations and only slight precipitates were formed in the preparations at high or low hydrogen-ion concentrations. The preparations were usually within 0.05 pH unit and never over 0.2 pH unit from the desired hydrogen-ion concentration at the end of the various time periods. The amounts of acid and alkali used to adjust and readjust the hydrogen-ion concentrations are not recorded, since they are only the amounts necessary to take care of the 0.1 M phosphate buffer. The virus and other materials present have no noticeable buffering capacity at the dilution used.

The results of the tests (Table 4) indicate that, in general, the rate of inactivation of virus in purified preparations is about the same as that of virus in diluted untreated juice except at low hydrogen-ion concentrations, such as pH 11 and pH 12, where the virus in purified preparations appears to be more stable. It may be seen from table 4 that the preparations at pH 9 and pH 10 still gave more than 50 per cent as many lesions as the control even at the end of 1 year. The purified preparation withstood momentary adjustment to pH 11 without appreciable loss of activity and even to pH 12 without complete loss of activity. Using the data given in table 5, it is possible to estimate the amount of virus remaining in the

TABLE 4.—*Tests for virus infectivity of a purified virus preparation at different hydrogen-ion concentrations immediately after adjustment and t pH 6.8, after standing at different hydrogen-ion concentrations at −14° C. for periods of time up to 1 year*

'repa-ations nain-ained ıt pH	Test plant	Preparations tested at different pH's immediately after adjustment		Preparations tested after an immediate readjustment to pH 6.8		Preparations tested at pH 6.8 after being held 1 hour at different pH's		Preparations tested at pH 6.8 after being held 2 days at different pH's		Preparations tested at pH 6.8 after being held 9 days at different pH's		Preparations tested at pH 6.8 after being held 32 days at different pH's		Preparations tested at pH 6.8 after being held 5 months at different pH's		Preparations tested at pH 6.8 after being held 12 months at different pH's	
		Averaged[a]	Actual[b]	Averaged	Actual	Averaged	Actual	Averaged	Actual	Averaged	Actual	Averaged	Actual	Averaged	Actual	Averaged	Actual
1	N. glutinosa	7.5	3.6	62.7	71.6	56.8	23.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	P. vulgaris	2.3	1.2	91.5	116.0	61.2	41.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2	N. glutinosa	35.6	11.7	84.7	59.6	114.7	58.8	65.5	81.4	7.9	5.9	0.0	0.0	0.0	0.0	0.0	0.0
	P. vulgaris	7.8	8.8	85.9	157.5	102.1	104.0	126.2	158.0	8.3	10.8	0.1	0.1	0.0	0.0	0.0	0.0
3	N. glutinosa	84.6	51.8	103.1	76.4	85.5	80.1	164.9	95.7	99.0	57.5	63.8	67.6	6.0	5.5	2.0	2.3
	P. vulgaris	27.4	19.4	98.2	133.2	100.8	139.0	143.0	149.1	95.0	93.0	74.0	148.0	2.9	2.3	1.6	3.1
4	N. glutinosa	85.0	51.4	98.2	82.1	144.0	59.0	133.0	87.7	94.0	58.5	92.3	107.0	128.0	90.4	89.1	88.4
	P. vulgaris	80.1	85.8	95.7	122.8	93.1	89.2	150.0	214.0	78.9	104.9	94.5	148.2	95.8	102.0	92.0	122.9
5	N. glutinosa	83.8	36.4	104.2	101.2	100.0	78.9	166.0	75.6	85.5	50.6	86.7	144.0	100.0	53.5	80.9	72.4
	P. vulgaris	93.0	100.0	110.3	142.3	101.8	95.9	136.0	179.0	80.0	106.5	80.4	142.0	109.0	141.5	86.5	105.8
6	N. glutinosa	118.0	44.1	101.3	89.8	108.9	55.0	109.2	86.4	102.0	114.7	67.1	147.8	103.0	68.8	91.6	81.2
	P. vulgaris	135.0	171.5	96.2	128.3	97.5	139.0	138.0	132.8	94.1	97.9	96.5	183.3	100.0	119.6	84.5	106.6
7	N. glutinosa	129.2	76.4	108.4	82.8	119.0	93.2	95.0	60.8	102.5	60.4	64.8	143.0	90.0	71.0	97.6	92.3
	P. vulgaris	140.0	219.8	89.2	132.1	139.5	137.2	137.0	133.0	81.0	124.0	73.0	156.0	115.0	179.5	83.5	106.2
8	N. glutinosa	128.4	42.4	96.3	84.1	116.0	82.0	128.4	72.5	95.8	74.4	72.6	131.0	74.3	51.3	83.1	76.7
	P. vulgaris	109.8	141.8	104.1	163.8	121.5	202.0	140.0	140.0	88.8	114.5	63.0	143.0	76.8	111.0	89.0	95.2
9	N. glutinosa	160.0	40.8	115.0	47.4	113.0	119.0	127.8	59.0	87.6	67.1	54.7	132.5	62.4	45.6	71.1	62.8
	P. vulgaris	132.4	216.0	76.5	182.2	93.0	77.0	128.5	108.0	83.4	111.5	63.3	187.8	84.6	82.0	96.5	87.5
10	N. glutinosa	80.0	60.3	76.3	64.1	92.5	97.2	70.5	29.2	67.6	65.0	61.2	76.5	53.2	25.6	46.3	31.8
	P. vulgaris	111.3	196.5	70.3	144.2	80.0	95.0	133.1	119.8	60.7	92.1	65.4	106.0	86.5	80.5	50.5	56.9
11	N. glutinosa	82.8	58.2	103.0	79.8	50.8	19.6	4.4	2.2	0.4	0.3	0.1	0.2	0.0	0.0	0.0	0.0
	P. vulgaris	112.0	151.3	90.1	124.7	50.1	56.6	5.0	5.3	0.4	0.5	0.1	0.1	0.0	0.0	0.0	0.0
12	N. glutinosa	0.0	0.0	1.1	1.3	1.3	0.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	P. vulgaris	0.0	0.0	0.2	0.2	1.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

[a] Numbers represent the quotient of the average number of lesions per half-leaf obtained on 5 or more half-leaves of *Nicotiana glutinosa* or 12 or ıore half-leaves of *Phaseolus vulgaris* on inoculation with the designated preparation, divided by the average number of lesions obtained on the other alves of the same leaves on inoculation with the control preparation.

[b] Numbers represent the average of the actual number of lesions per half-leaf obtained on 5 or more half-leaves of *N. glutinosa* or 12 or more half-eaves of *P. vulgaris* on inoculation with the designated preparation.

various samples. The rate of inactivation of virus in the purified samples is so slow as to be negligible at pH 4 to pH 8, very slow at pH 9 and pH 10, fairly rapid at pH 2, pH 3 and pH 11, and very rapid at pH 1 and pH 12. The virus preparations which were completely inactivated at pH 1, pH 2, pH 11, and pH 12 and then adjusted to pH 6.8 were again tested for virus infectivity after standing for periods of time as long as 1 year, and in no instance was it possible to demonstrate the presence of active virus.

Inactivation of Tobacco-ring-spot and Cucumber-mosaic Viruses at Different Hydrogen-ion Concentrations[2]

Since tobacco-mosaic virus is more stable, in many respects (10), than other viruses, such as tobacco-ring-spot and cucumber-mosaic, it seemed desirable to determine, for purposes of comparison, whether these two viruses are more or less stable at different hydrogen-ion concentrations than tobacco-mosaic virus. Experiments were made, therefore, to determine the effect of standing at different hydrogen-ion concentrations for 1 hour at room temperature on the inactivation of tobacco-ring-spot (Wingard strain) and cucumber-mosaic virus.

Infectious juice from plants of *Nicotiana tabacum* variety Turkish infected with ring-spot virus was diluted with 4 parts of distilled water and 50 cc. portions were taken to pH 2 to pH 12 in 1 pH gradations by the addition of 2 N NaOH or HCl with vigorous stirring. The prepara-

TABLE 5.—*Tests for virus infectivity of dilutions of control preparations*

Dilution	Tested on *Phaseolus vulgaris*		Tested on *Nicotiana glutinosa*	
	Diluted[a] untreated juice	Purified[b] virus preparation	Diluted[a] untreated juice	Purified[b] virus preparation
1	67.5[c]	161.0[d]	107.0[c]	179.0[d]
1×10^{-1}	20.1	125.3	17.7	43.8
1×10^{-2}	3.5	74.8	3.4	12.9
1×10^{-3}	0.2	24.0	1.0	7.5
1×10^{-4}	0.4	8.1	0.0	1.4

[a] Dilutions were made with distilled water.

[b] Dilutions were made with 0.1 M phosphate at pH 6.8.

[c] Numbers represent the average number of lesions per half-leaf obtained on the left halves of leaves on inoculation with the designated preparation and dilution.

[d] Numbers represent the average number of lesions per half-leaf obtained on the right halves of the same leaves on inoculation with the designated preparation and dilution.

[2] These experiments were made in collaboration with Dr. W. C. Price, who carried out the tests for virus infectivity.

tions at the 10 different hydrogen-ion concentrations were tested immediately after adjustment for virus infectivity on cowpea, *Vigna sinensis* (L.) Endl. var. Black Eye. After standing for 1 hour at room temperature (20° C.), the preparations were readjusted to the hydrogen-ion concentration of the untreated preparation (pH 6.02) and again tested for virus infectivity. The results of the tests (Table 6) show that the virus is completely inactivated after standing for 1 hour at pH 2, pH 10

TABLE 6.—*Tests for virus infectivity on cowpea of a preparation of tobacco-ring-spot virus at different hydrogen-ion concentrations immediately after adjustment and at pH 6 after standing for 1 hour*

Preparations held at pH →	2	3	4	5	6	7	8	9	10	11
Preparations tested at different pH's immediately after adjustment	0.00[a]	0.00	0.05	0.35	9.40	14.10	19.70	5.39	0.00	0.00
Preparations tested at pH 6 after being held 1 hour at different pH's	0.00	0.10	1.05	2.40	11.30	5.29	6.50	0.32	0.00	0.00

[a] Numbers represent the average number of lesions per leaf obtained on 40 leaves of cowpea, *Vigna sinensis* var. Black Eye, on inoculation with the designated preparation.

and pH 11. It is also almost completely inactivated after standing for an hour at pH 3 or pH 9. The results indicate that the virus tends to inactivate as the hydrogen-ion concentration is increased or decreased from pH 6. The increased number of lesions produced on immediate inocula-

TABLE 7.—*Tests for virus infectivity on cowpea of a preparation of cucumber-mosaic virus at different hydrogen-ion concentrations immediately after adjustment and at pH 5.7 after standing for 1 hour*

Preparations held at pH →	Dilution[a]	3	5	5.7	7	9
Preparations tested at different pH's immediately after adjustment	Undiluted	0.17[b]	3.19	1.87	2.85	1.85
	1×10^{-1}	0.00	0.43	0.30	0.40	1.12
	1×10^{-2}	0.00	0.00	0.05	0.00	0.06
Preparations tested at pH 5.7 after being held 1 hour at different pH's	Undiluted	0.19	1.58	2.94	1.67	0.63
	1×10^{-1}	0.00	0.17	0.31	0.28	0.07
	1×10^{-2}	0.00	0.00	0.00	0.00	0.00

[a] Dilutions were made with distilled water.

[b] Numbers represent the average number of lesions per leaf obtained on 40 leaves of cowpea, *Vigna sinensis* var. Black Eye, on inoculation with the designated preparation.

tion at pH 7 and pH 8 and the decreased number at pH 5, pH 4 and pH 3 is a phenomenon similar to that encountered with tobacco-mosaic virus on *Phaseolus vulgaris*.

Portions of 30 cc. each of infectious juice at pH 5.7 from plants of *Nicotiana tabacum* variety Turkish infected with ordinary cucumber-mosaic were taken to pH 3, pH 5, pH 7 and pH 9 and immediately tested with the untreated control for virus infectivity on cowpea, *Vigna sinensis* variety Black Eye. After standing for 1 hour at 20° C., the preparations were readjusted to pH 5.7 ± 0.1 and again tested for virus infectivity. The results, which are given in table 7, are in general similar to the results obtained with ring-spot virus. The cucumber-mosaic virus is inactivated as the hydrogen-ion concentration is increased or decreased from pH 5.7. Ring-spot and cucumber-mosaic viruses are, therefore, much less stable than tobacco-mosaic virus as the hydrogen-ion concentration is increased or decreased from about pH 6.

DISCUSSION

The experiments described in this paper show that the rate of inactivation of tobacco-mosaic virus in diluted untreated infectious juice at room temperature or $-14°$ C. is so slow as to be negligible between pH 3 and pH 8, fairly rapid between pH 1.5 and pH 2.5 and between pH 9 and pH 10, and very rapid between pH 0.5 and pH 1.5 and between pH 11 and pH 12.

It is sometimes desirable, in making a comparative study of the properties of a virus, to be able to remove different amounts of virus from a given preparation in order to study the properties of the remaining virus. This may be accomplished easily by adjusting virus preparations to hydrogen-ion concentrations at which the rate of inactivation is fairly rapid and readjusting portions of the preparation to a favorable hydrogen-ion concentration after different periods of time. Any desired percentage of the original amount of virus may be obtained by adjusting the hydrogen-ion concentration and the time of exposure. It should be noted that the amount of acid or alkali necessary to adjust a virus sample to a given hydrogen-ion concentration varies with the buffering capacity of the virus preparation. This may vary considerably, depending largely upon the method used to prepare the virus sample. In general, preparations containing large amounts of solids have a greater buffering capacity and require more acid or alkali than preparations that have small amounts of solids. The data concerning the amounts of acid or alkali used, which are recorded in table 1, are valid, therefore, only for virus samples prepared as described in the first experiment. Even then, some variation may be expected with samples prepared at different times from different sets of

plants. The absence of the large amount of organic solids ordinarily present in untreated juice may be responsible for the fact that the rate of inactivation of virus in purified preparations is somewhat greater at pH 3 and somewhat less at pH 9, pH 10, and pH 11.

The fact that it was not found possible to demonstrate active virus in preparations completely inactivated at unfavorable hydrogen-ion concentrations, even after long standing at favorable reactions, indicates that such treatment does not cause inactivated virus to become active again. The reactivation experiment described by Brewer, Kraybill, Samson, and Gardner (3) could not be repeated, inasmuch as it was found impossible to inactivate virus completely at pH 8.5.

One factor, the importance of which has apparently been overlooked by many workers and that may be responsible for apparent discrepancies, is the effect of the hydrogen-ion concentration of virus preparations at time of inoculation on the infectivity of virus. It may be seen from table 3 that *Nicotiana glutinosa* was quite susceptible to virus at pH 2, pH 2.5, pH 3.0, and pH 3.5, whereas *Phaseolus vulgaris* gave only about 10 per cent. as many lesions at these high hydrogen-ion concentrations as at pH 6. However, *P. vulgaris* gave more lesions with virus at low hydrogen-ion concentrations such as pH 9.5, pH 10, and pH 10.5 than it did with virus preparations at pH 6. The actual number of lesions obtained with a virus preparation on inoculation to *P. vulgaris* may be increased or decreased remarkably by a change in the hydrogen-ion concentration of the virus preparation. For example, it may be seen from table 3 that the preparation at pH 1.5 gave an average of only 0.3 lesions per half-leaf on *P. vulgaris* or 1.1 per cent. as many as the control, whereas another portion of the same virus preparation at pH 10.0 gave 121.0 lesions per half-leaf or 485 per cent. as many as the same control, an increase of over 400 times. This increase is not due to inactivation of virus at pH 1.5, since it may be seen from table 3 that each preparation when brought to pH 6 gave about as many lesions as the untreated control. It is possible that there is a tendency for virus to aggregate at high hydrogen-ion concentrations and to become dispersed at low hydrogen-ion concentrations, and thus cause a change in the actual number of infective units present. This can not be responsible for much of the change, for, as may be seen from table 3, virus preparations at pH 2 and pH 10 still gave about the same number of lesions on *N. glutinosa* as the control. This is an indication that the number of infective units is about the same in either case. It seems likely, therefore, that most of the change is due to the effect of the hydrogen-ion concentration of the virus preparation on the cells of the plant used to measure the virus infectivity. Cells of plants of *N. glutinosa* are apparently little

affected by quite large changes in hydrogen-ion concentration, whereas cells of *P. vulgaris* plants are adversely affected, as regards susceptibility to virus, at high hydrogen-ion concentrations such as pH 1 to pH 3.5, and beneficially affected at low hydrogen-ion concentrations such as pH 9.5 to pH 10.5. In further confirmation of this point, it may be seen from table 3 that virus preparations at pH 1 and pH 11.5 gave no lesions on *P. vulgaris,* yet gave a few lesions on *N. glutinosa*. *Vigna sinensis* plants are affected by changes in the hydrogen-ion concentration of the inoculum similarly to those of *P. vulgaris.*

SUMMARY

The rate of inactivation of tobacco-mosaic virus in diluted untreated infectious juice of *Nicotiana tabacum* L. variety Turkish held at 20° C. or – 14° C. is so slow as to be negligible between pH 3 and pH 8, fairly rapid between pH 1.5 and pH 2.5 and between pH 9 and pH 10, and very rapid between pH 0.5 and pH 1.5 and between pH 11 and pH 12. The rate of inactivation of virus in purified virus preparations is similar except that the rate is somewhat greater at pH 3 and somewhat less between pH 9 and pH 11.

Reactivation of virus completely inactivated at pH 1, pH 2, pH 11, or pH 12 was not observed.

The susceptibility of plants of *Nicotiana glutinosa* L. to tobacco-mosaic virus inoculated at reactions from pH 2 to pH 10.5 did not change greatly. Plants of *Phaseolus vulgaris* L. variety Early Golden Cluster were much more susceptible to virus inoculated at reactions of from pH 9 to pH 10.5 than to virus inoculated at pH 0.5 to pH 3.

Tobacco-ring-spot and cucumber-mosaic viruses were much less stable than tobacco-mosaic virus, inactivation increasing as the hydrogen-ion concentration was increased or decreased from about pH 6.

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INFLUENCE OF PHOSPHORUS AND POTASSIUM SUPPLY ON HOST SUSCEPTIBILITY TO YELLOW TOBACCO MOSAIC INFECTION[1]

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Host nutrition has long been recognized by plant pathologists as an important factor in modifying host susceptibility to parasitic infection. Up to the present time, however, little attention has been given to this factor in most plant-virus investigations. The effect of nitrogen supply on host susceptibility to infection with tobacco mosaic has already been considered in a previous paper (8). The object of the present paper is to report the results of further experiments in which the effect of phosphorus and potassium supply on the susceptibility of tobacco to virus infection was studied.

REVIEW OF LITERATURE

A review of the plant-virus literature reveals only a very limited number of papers dealing with the effect of phosphorus and potassium supply on host susceptibility to virus infection. Schultz and Folsom (7) were among the first to consider host nutrition in connection with plant viruses. They found that the percentage of mosaic and the distinctness of mottling on Green Mountain potatoes were reduced by high nitrogen and high potassium fertilization during the first part of the season. Janssen (3), in a series of fertilizer tests with nitrogen, phosphorus, and potassium on two different types of soils, observed that the incidence of mosaic on potato was highest in plots deprived of potassium. Quanjer (5) concluded from his investigations and those of his collaborators that potassium retards the development and spread of pseudo-net-necrosis to healthy potato plants.

Ainsworth (1), in studies on permanent soil plots receiving the same treatment each year, observed that the number of tomato plants affected with tomato stripe on plots receiving a complete fertilizer was 5.0 per cent. However, when potassium was omitted from the fertilizer mixture the number of diseased plants increased to 23.3 per cent. Omission of phosphate lowered the percentage only slightly.

Volk (9), growing tomato and tobacco plants in nutrient sand cultures, obtained some interesting results on the effect of nutrition on host susceptibility to spot-mosaic virus (Fleckmosaik). He reports that susceptibility

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of tomato is not influenced noticeably by variations in the potassium supply, but is decreased when phosphorus is withheld. Tobacco plants were inoculated at the preblooming stage by injecting expressed sap under the epidermis of the lowermost leaf on each plant. The various amounts of potassium and phosphorus used exerted no change in susceptibility of tobacco to infection, susceptibility being measured by the number of plants that became diseased. These results with regard to potassium are not in accord with those found by the other workers previously mentioned.

MATERIALS AND METHODS

In the experiments here reported, *Nicotiana tabacum* L. var. Turkish was used as the host plant. Growth was measured by determining both the green weights and the heights of representative plants in each test at time of inoculation. Only green-weight data will be presented, however, since the resultant graphs of these two growth criteria proved to be similar. Susceptibility to infection was measured by counting the number of primary lesions for each 100 pin punctures.

Tobacco seedlings were germinated in white quartz sand and grown in this medium for about 3 weeks, during which time they were kept moist with a dilute complete nutrient solution. When the seedlings had developed 3 or 4 small leaves and were about 2 cm. in height, uniform plants were selected and potted in 4-inch porous clay pots in a soil of low fertility. The pots were then placed in saucers on a bench in a greenhouse and were spaced sufficiently to prevent undue crowding of foliage. While the experiments were in progress the greenhouse temperature was held between 70° and 80° F. during the day and between 70° and 75° F. at night. The greenhouse was fumigated at weekly intervals to guard against possible insect transmission of the mosaic.

Nutrient treatments were started 3 or 4 days after the seedlings had been potted. The plants were grouped into several different treatments with 10 pots in each. Twice a week each plant received 100 cc. of a basic nutrient solution, to which the calculated amounts of phosphorus and potassium were added, as will be discussed later.

The virus used, a yellow tobacco-mosaic virus, designated by Johnson (4) as tobacco virus 6, was obtained from James Johnson several years ago. Since that time it has been carried through repeated transfer in tobacco plants. This virus was chosen as the infective agent because of the conspicuous primary lesions that it produces on tobacco. These lesions usually become visible as small, yellowish areas on the 3rd day after inoculation. They may be more easily distinguished if the leaves are first decolorized in warm alcohol and then stained in a weak potassium iodide-iodine solution, as described by Samuel (6). The primary lesions, in which starch

formation is inhibited completely, show up as clear spots against the dark background of healthy starch-filled tissue. Figure 1 shows a typical tobacco leaf after such treatment on the 8th day following inoculation.

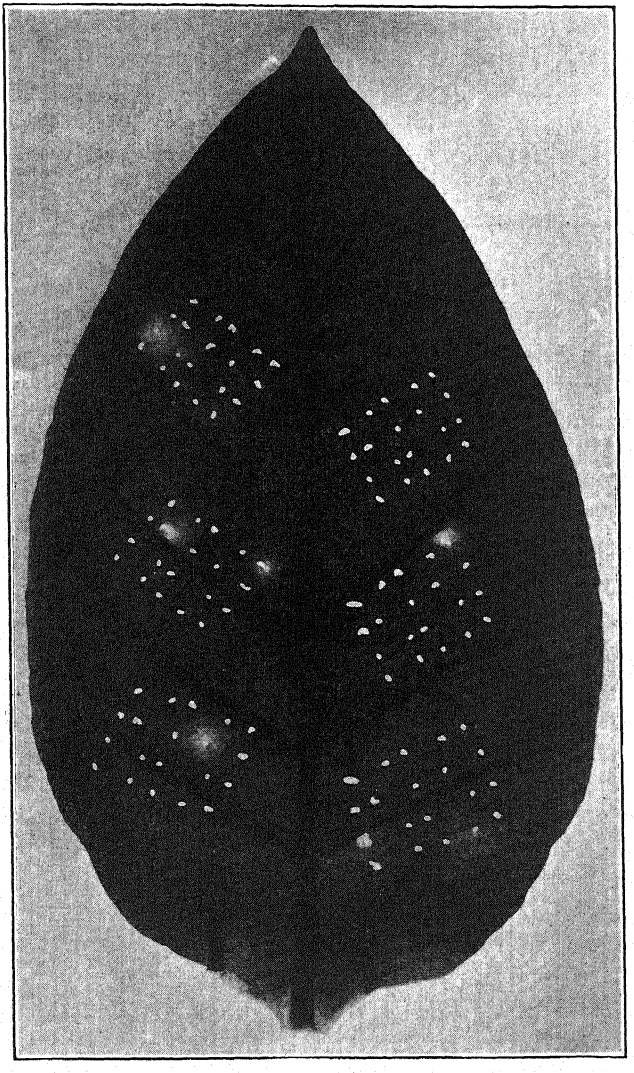


FIG. 1. Primary lesions of yellow tobacco mosaic on tobacco after the leaf has been stained with iodine. The primary lesions appear as clear areas against the dark background.

When the majority of the plants had formed 12 or 13 leaves, representative plants were inoculated. The pin-puncture method, as described

by Holmes (2), was used in order to introduce relatively uniform doses of inoculum. A set of 20 small insect pins, size No. 000, were bound between pot labels, so that 20 pricks could be made with each thrust. The number of punctures per leaf ranged from 60 to 260, depending on the size of the leaf. In these experiments only leaves in the upper and middle portion of each plant were inoculated, since data presented in an earlier paper (8) showed that results obtained from leaves on the upper and middle portion of a plant were similar to those obtained when all the leaves were inoculated. The pins were dipped into undiluted juice from a diseased plant each time before being pricked into the test plant. All experiments have been repeated one or more times.

INFLUENCE OF PHOSPHORUS SUPPLY ON SUSCEPTIBILITY TO INFECTION WITH YELLOW TOBACCO MOSAIC

The basic nutrient solution used in the phosphorus tests was made up of a $MgSO_4$ and $CaCl_2$ solution to which nitrogen, potassium and phosphorus were added. In the 10 treatments phosphorus was added in the form of KH_2PO_4 and $NH_4H_2PO_4$ in doses ranging from 0 to 150 mg. of phosphorus per plant per day. The potassium concentration of all solutions was held constant at 100 mg. per plant by adding the calculated amounts of K_2SO_4. The nitrogen concentration was held at 31 mg. per plant by adding the required amounts of $(NH_4)_2SO_4$. After 3 weeks of nutrient treatment the plants were large enough to be inoculated.

Figure 2 shows representative plants that received 0, 5, 20, 60, and 150 mg. of phosphorus per day, respectively, for 25 days, photographed at time of inoculation. Growth was not greatly retarded even when 150 mg. of phosphorus per day were added. The color of all the plants was similar

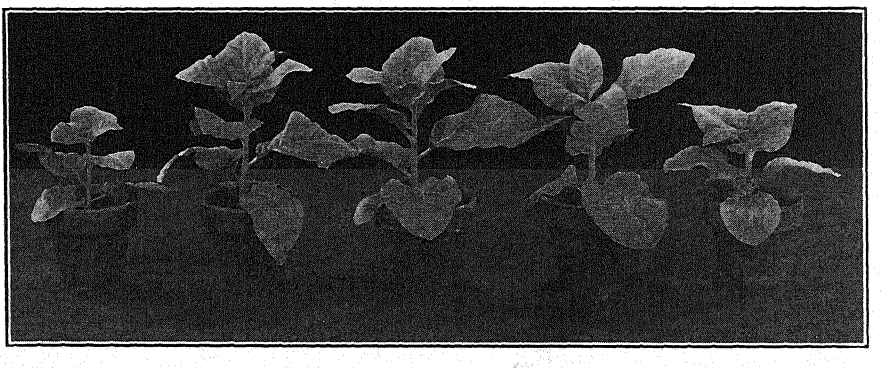


FIG. 2. Typical tobacco plants that received 0, 5, 20, 60, and 150 mg. of phosphorus per day, respectively. The middle plants were nearly equal in susceptibility.

except in the case of those receiving no phosphorus. The latter were a dark olive green.

TABLE 1.—*Effect of phosphorus supply on growth and susceptibility of tobacco to yellow tobacco mosaic*

Phosphorus added per day (*mg.*)	Green weight at time of inoculation (*gm.*)	Number of		
		Pin punctures	Lesions	Lesions per 100 pin punctures
0	19[a]	8300[b]	366[b]	4.4
5	41	12640	1391	11.0
10	40	13600	1518	11.2
20	41	13600	1551	11.4
40	41	13600	1468	10.8
60	39	13600	1423	10.5
80	41	13600	1305	9.6
100	36	12400	1153	9.3
125	36	12400	1054	8.5
150	26	9700	743	7.7

[a] Average of 10 plants.
[b] Total number on 10 plants.

In table 1 are recorded the composite results of two tests on the influence of phosphorus supply on growth and susceptibility to infection. The green-weight data at time of inoculation represent the average of 10 plants for each treatment. These data show that equally good growth was produced by varying amounts of phosphorus (5–80 mg. of phosphorus per day). With larger doses of phosphorus, total growth of the plants was less. From this table it is also evident that several different doses of phosphorus gave equally high susceptibility (5–60 mg. of phosphorus per day), although susceptibility was gradually lowered when more than 20 mg. of phosphorus were added per day.

The influence of phosphorus supply on growth and susceptibility is expressed graphically in figure 3. The solid line represents total growth as measured by green weights of representative plants at time of inoculation. The broken line represents susceptibility to infection as measured by the number of lesions per 100 pin punctures. These graphs show that susceptibility was correlated directly with growth. That is to say, susceptibility increased when growth increased, and decreased when growth fell off due to excess phosphorus. It is probable that this variation in susceptibility was due primarily to variation in growth and only indirectly to the phosphorus supply.

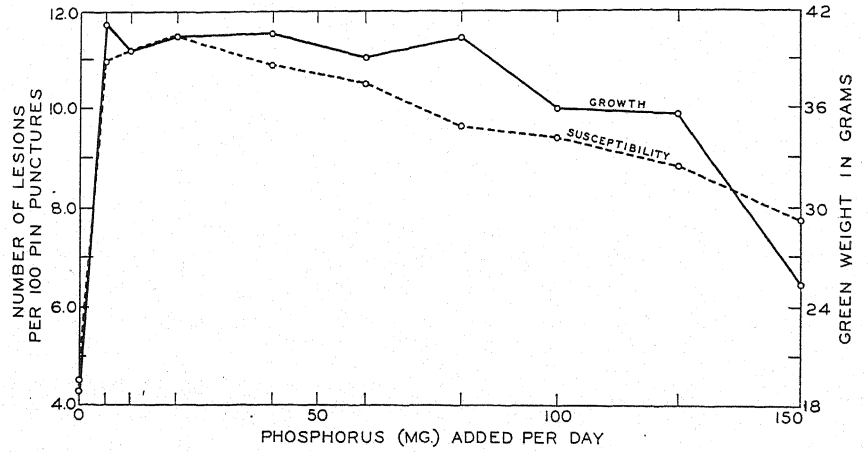


FIG. 3. Influence of phosphorus supply on growth and susceptibility of tobacco to yellow tobacco mosaic. The solid line represents green weight of plants; the broken line, the number of lesions per 100 pin punctures.

INFLUENCE OF POTASSIUM SUPPLY ON SUSCEPTIBILITY TO INFECTION WITH YELLOW TOBACCO MOSAIC

In the potassium tests the doses received by the plants in the 11 treatments ranged from 0 to 500 mg. of potassium, in the form of K_2SO_4, per plant per day. The supply of the other essential elements was held constant in all treatments. Each plant received 14.6 mg. of nitrogen and 10.0 mg. of phosphorus with every feeding of potassium. Small amounts of calcium and magnesium also were added. The plants were inoculated 30 to 34 days after potassium treatments were started.

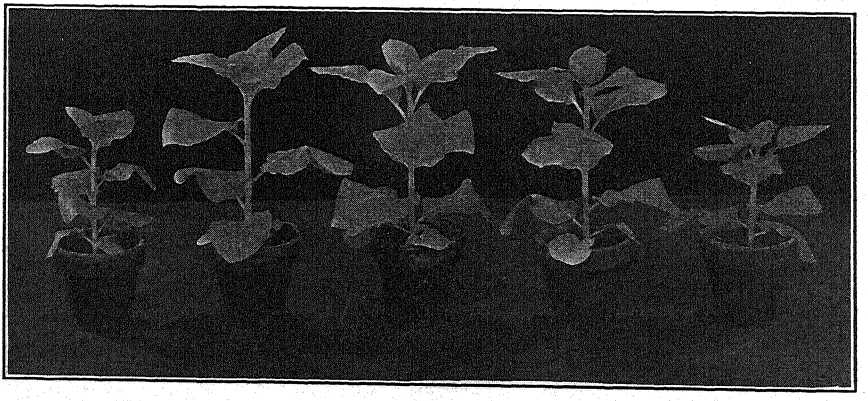


FIG. 4. Typical tobacco plants that received 0, 20, 100, 150, and 300 mg. of potassium per day, respectively, from left to right, photographed at time of inoculation. The second plant from the left was the most susceptible.

Figure 4 shows typical tobacco plants that received 0, 20, 100, 150, and 300 mg. of potassium per day, respectively, photographed at time of inoculation. No symptoms of toxicity were noticed in any of the treatments when less than 400 mg. of potassium per day were added. Above this dose, the plants became dwarfed and made very little growth. When no potassium was added, the foliage became conspicuously mottled and yellowish in color. The oldest leaves in this treatment were spotted with white necrotic areas where tissue had broken down.

The composite results of three tests on the influence of potassium supply on growth and susceptibility to infection are given in table 2. From the

TABLE 2.—*Effect of potassium supply on growth and susceptibility of tobacco to yellow tobacco mosaic*

Potassium added per day (*mg.*)	Green weight at time of inoculation (*gm.*)	Number of		
		Pin punctures	Lesions	Lesions per 100 pin punctures
0	15[a]	15000[b]	379[b]	2.5
10	26	13100	585	4.5
20	26	19600	931	4.8
40	27	19600	712	3.6
70	27	19600	633	3.2
100	27	18640	644	3.5
150	24	18640	577	3.1
200	23	17060	401	2.4
300	16	12200	291	2.4
400	7	3140	48	1.5
500	4	1500	17	1.1

[a] Average of 15 plants.

[b] Total number on 15 plants.

green-weight data, which represent averages of 15 plants on each treatment, it is evident that equally good growth was produced with a wide range of potassium dosages (10–150 mg. of potassium per day). Beyond this range growth fell off gradually until, at 500 mg. per day, growth was retarded to such an extent that most of the plants died. From this table it also is apparent that susceptibility, as measured by the average number of lesions per 100 pin punctures, was highest with very low doses of potassium and decreased when the size of the dose of potassium was increased.

In figure 5 the relation between growth and susceptibility as influenced by potassium supply is expressed graphically. The solid line represents average green weights of representative plants at time of inoculation. The broken line represents the average number of lesions per 100 pin punctures. These graphs show that good growth was produced by the addition of

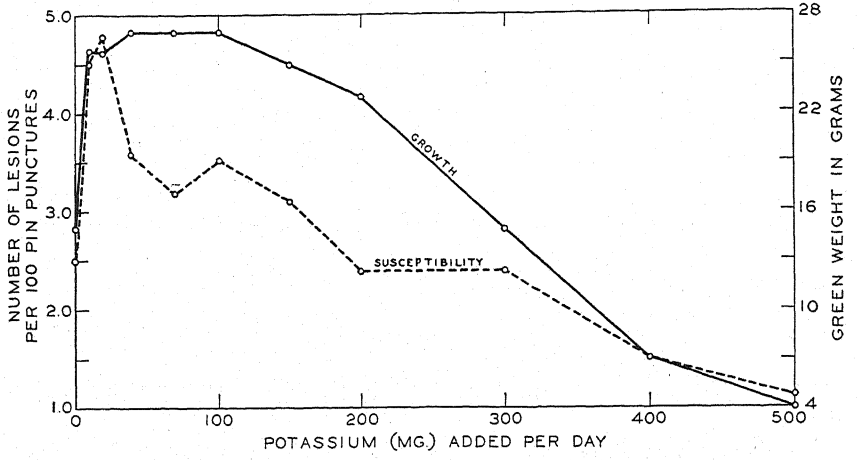


FIG. 5. Relation between growth and susceptibility of tobacco to yellow tobacco mosaic as influenced by potassium supply. The solid line represents green weight of plants; the broken line, the number of lesions per 100 pin punctures when potassium is added as indicated along the base line.

from 10 to 150 mg. of potassium per day. High susceptibility, on the other hand, was limited to very small doses of potassium, between 10 and 20 mg. per day. Below and above this restricted range, susceptibility fell off markedly. Thus, it is evident that host susceptibility to infection was influenced more by potassium than it was by growth, since doses of potassium between 20 and 150 mg. per day decreased susceptibility pronouncedly, whereas growth remained constant.

DISCUSSION

Data obtained in this investigation indicate that phosphorus supply has apparently only an indirect influence on susceptibility of tobacco to infection with yellow tobacco mosaic, inasmuch as susceptibility and growth seem to be correlated directly with each other. Potassium supply, on the other hand, has a direct influence in changing host susceptibility to infection. When more than 20 mg. of potassium were added each day, susceptibility decreased appreciably. Growth, however, was not retarded noticeably until the addition of potassium exceeded 200 mg. per day.

These experiments do not permit the formulation of any definite explanation of the effect of potassium and phosphorus supply on host susceptibility to parasitic infection. Although it is concluded that host susceptibility has been altered by mineral nutrition, further evidence is needed to show whether certain of the mineral elements, or some substances dependent on a specific mineral nutrition for their synthesis, are the factors determining the degree of susceptibility of the host. This evidence can

be obtained only by an investigation of the physiological and morphological conditions that exist within the plant itself.

The physiological rôles of potassium and phosphorus in plants have been studied by many plant physiologists. The effect of deficiencies of these particular elements on the histological structure and organic constituents of plants also has been investigated, but the effect of excess potassium and phosphorus on plant organisms is still a matter of question. An explanation of the apparent tolerance of tobacco to large amounts of potassium and phosphorus without any visible change in growth response is difficult. It has long been known by soil scientists that vegetative and reproductive conditions of plants are not changed appreciably by the "luxury consumption" of salts of potassium, phosphorus, and other minerals. It may be that the response of tobacco to large amounts of these elements can be explained on this basis.

The conclusion that potassium decreases susceptibility in tobacco substantiates the findings of several investigators with other plant viruses, but is not in accord with those published by Volk (9), who found that potassium and phosphorus exerted no influence on the susceptibility of tobacco.

SUMMARY

The influence of phosphorus and potassium supply on growth and susceptibility of tobacco to yellow tobacco mosaic was studied.

Plants grown in soil with various doses of potassium and phosphorus were inoculated by the pin-puncture method with yellow tobacco-mosaic virus (Johnson's tobacco virus 6). Growth was measured by green-weight determinations at time of inoculation, and susceptibility to infection by the number of lesions per 100 pin punctures.

In the phosphorus study susceptibility was correlated directly with growth. It is probable that this variation in susceptibility was due primarily to variations in growth and thus only indirectly to the phosphorus supply.

Results from the study of potassium indicate that host susceptibility was influenced more by potassium supply than it was by growth. Growth was favored by small and medium doses of potassium. Susceptibility to infection, however, was increased by very small doses, but was decreased markedly by moderately larger doses. Thus, susceptibility to infection can be lowered without retarding growth by increasing the supply of potassium within certain limits.

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INFECCIÓN EXPERIMENTAL DEL CENTENO DE PETKUS (SECALE CEREALE V. VULGARE), POR LAS CARIES DEL TRIGO: TILLETIA TRITICI Y TILLETIA LEVIS

RAIMUNDO NIEVES, ING. AGR.[1]

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Durante 3 años, 1931, 1932 y 1933, he obtenido experimentalmente *Tilletia tritici* (Bjerk.) Wint. y *Tilletia levis* Kühn, sobre *Secale cereale* v. *vulgare*.

Solo ciertas formas fisiológicas de estos hongos, parecen capaces de atacar a este nuevo huésped, siendo la primera vez que se cita al centeno de Petkus (variedad oriunda de Alemania) como infectado por ambas Tilletias del trigo. Por otra parte, las comunicaciones sobre este género de parasitismo, son sumamente escasas.

He creido conveniente no demorar mas la comunicación de estos resultados[2] por cuanto los ensayos de 1931 y 1932, son absolutamente convincentes, en el sentido de evidenciar al centeno como huésped de dos hongos que en la literatura corriente y durante muchos años se han creido parásitos estrictos del trigo.

ANTECEDENTES EN LA LITERATURA AGRÍCOLA

El centeno es atacado raramente por una carie específica, la *Tilletia secalis* (Cda.) Kühn, señalada para Europa desde hace muchos años (14). Sus esporos son análogos a los de *Tilletia tritici*. En Estados Unidos ha sido señalada por Kirby (12); pero tambien como de aparición muy rara. Kirby dice: "La carie del centeno (*Tilletia secalis*) ha sido encontrada una vez en New York. El centeno es muy resistente, y nunca se ha encontrado mas que unas pocas espigas infectadas. La carie del trigo comunmente encontrada en este Estado, es causada por otra especie de hongo (*T. tritici*). Si este hongo ataca al centeno es desconocido." Ignoro si en nuestro país ha sido señalada la *Tilletia secalis*, pero creo probable que no.

La aparición espontánea de la caries del trigo sobre el centeno, o su inoculación experimental, son aparentemente hechos recien establecidos. Vavilov en su clásica monografía (25) al dar la lista de los géneros

[1] Jefe de la Sección Fitotecnia del Ministerio de Agricultura de la Nación.

[2] The writer wishes to thank Dr. H. B. Humphrey for his hearty cooperation during the progress of this work and for helpful suggestions in the preparation of the manuscript.

y especies de cereales atacados por *Tilletia tritici,* consigna únicamente el género *Triticum* sp. y *Aegilops* sp. No hace referencia alguna sobre *T. levis.* Ignora al centeno como huésped de la *T. tritici,* pero lo señala como huésped de *Ustilago tritici* (Pers.) Rostr., habiéndolo encontrado el autor en el norte de Persia, cerca de Hamadan, en un campo de trigo, en el que el centeno era espontáneo. Delacroix y Maublanc (4), Voglino (26) y Stevens and Hall (24) en sus manuales de Patología Vegetal, ignoran al centeno como huésped de las Tilletias del trigo. Según Heald (9) la primera información parece pertenecer a Gaines y Stevenson (1923) quienes producen experimentalmente en Pullman (Washington), *Tilletia tritici* sobre centeno e híbridos de trigo por centeno. Johnston[3] en Kansas, infectando experimentalmente centeno con *Tilletia* sp. (probablemente *T. levis*), encuentra algunas espigas infectadas, sobre varios miles de espigas examinadas.

Bressman (1) patólogo de la Oregon Agr. Exp. Sta., inocula en 1930 los centenos Oregon Sel. 1 y Rosen, con 31 colecciones de caries. Solo la *T. tritici* f.f. 9 infectó ambos centenos. Se examinaron 4,000 espigas, en los ensayos realizados en Corvallis. En material duplicado sembrado en otro lugar de Oregon (en Pendleton), Foster Martin encuentra que ambas Tilletias infectan el centeno, pero aquí tambien la *T. tritici* f.f. 9, se manifestó la más virulenta. Bressman concluye su trabajo sugiriendo si la *T. secalis* (Cda.) Kühn, no sería una forma fisiológica de *T. tritici* y debiera ser designada como tal. Si las Tilletias del trigo tienen mas huéspedes que los citados, no creo que esté bien establecido en la literatura corriente.

Voglino (26) refiriéndose aparentemente a la *T. tritici,* dice de un modo ambiguo: "La carie (*Tilletia caries* Tulasne), infesta le pianticine di grano ed un gran numero di graminacee selvatiche." La *T. levis* la consigna como específica del trigo.

Recientemente Bressman (3), consiguió inocular experimentalmente *T. levis* sobre *Lolium multiflorum* y *Lolium perenne,* consiguiendo 0.5 a 1 por ciento de infeccion. En nuestro país no se había señalado el parasitismo espontáneo o experimental de las Tilletias del trigo sobre el centeno, hasta la aparición de mi trabajo "La caries del trigo," (15) donde trato este asunto de manera incidental. Pero esta monografía es la primera, donde considero el tema con suficiente amplitud.

MATERIAL Y MÉTODOS

El centeno de Petkus, me fué proporcionado por el Ingº R. Bence Pieres, en 1926, quien lo recibió del Dr. Restelli, entonces Subsecretario del Ministerio de Relaciones Exteriores. Las colecciones de *Tilletia* sp. han sido recogidas por mí, en giras por las chacras, y otras solicitadas a diversos

[3] Johnston, C. O. U. S. Dept. Agr. Plant Dis. Reptr. Suppl. 62: 324. 1928.

colegas. Sobre 43 procedencias testadas en 1933, 34 las he colectado personalmente. Me fueron provistas: las colecciones 14, 15 y 17, por el Ingº Domingo Dávila; la 21 por el Ingº Santiago Boaglio; la 22 por el Agrº Williamson; la 24 por el Ingº Vicente C. Brunini; la 25, 26, 27 y 28 por el Dr. Wilhem Rudorf. Agradezco a mis distinguidos colegas su colaboración.

Todas las procedencias corresponden a La Pampa y Provincia de Buenos Aires, excepto las provistas por el Dr. Rudorf, que corresponden a Alemania. Las inoculaciones experimentales, se realizaron empolvando hasta el ennegrecimiento la semilla de los huéspedes (trigos o centeno), poco antes de la siembra, con clamidósporos frescos (del año anterior) de las *Tilletia* spp. Como es lógico, se han evitado posibles contaminaciones entre colecciones.

En cada ensayo se hicieron siembras individuales de los granos, comunmente a 7.5 × 20 cms. y a veces a 15 × 15 cms. Comunmente destinaba una parcela de 180 plantas para cada variedad. Los operadores se lavan las manos con soluciones de formol, despues de manipular cada colección. La época de siembra preferida fué a fines de Abril o principios de Mayo, pues entonces tenemos en Guatraché temperaturas óptimas para conseguir pesadas infecciones. Por otra parte la humedad del suelo y la contextura fina y friable de este último, son normalmente óptimos para la mejor germinación de las Tilletias.

Despues de la maduracióon de los trigos y centeno, se arrancan las plantas, se engavillan, etiquetan, etc. y luego se analizan. El recuento de las espigas infectadas, se hace pellizcando las espigas en dos o tres partes, a fin de poner en evidencia si tiene granos sanos o cápsulas de carbón (smut

TABLA Nº 1.—*Resultados de la infección experimental del centeno por 6 colecciones de T. tritici y 2 de T. levis, en el año 1931–32*

Siembra: Abril 23	Colecciones de *T. tritici*						Colecciones de *T. levis*		Totales
	2	3	5	6	7	9	1	8	
Procedencia	L.M.	Pel.	21	G	G	G	L.	21	
Nº espigas	789	930	937	738	916	677	752	1022	6761
Esp. infec.	—	15	—	8	—	16	—	29	68
(Por ciento)	0	1.7	0	1.1	0	2.4	0	2.9	10 o/oo
Siembra: Julio 20									
Nº espigas	922	1089	1203	918	1144	892	1277	1222	8667
Esp. infec.	—	—	—	2	—	—	—	—	2
(Por ciento)	0	0	0	0.2	0	0	0	0	0.2 o/oo

balls). Para el personal especializado basta generalmente ver la espiga para juzgar si está sana o enferma. Pero en los casos dudosos o con personal poco familiarizado se utiliza siempre el método del "pellizcado."

En los ensayos que consigno en las tablas N° 1 y 2, se han examinado 39.327 espigas de centeno. En la tabla 3; 53.534 espigas de trigo.

RESULTADOS

En 1931, en un ensayo sobre especialización fisiológica de la caries del trigo (siembra temprana), en el que estudiaba la patogenia de colecciones de *Tilletia* sp. sobre 18 huéspedes diferenciales, conseguí vencer la inmunidad natural del centeno de Petkus, obteniendo 68 espigas infectadas, sobre 6.761 examinadas—10 por mil—(véase tabla n° 1). Las colecciones n° 3; 6 y 9 de *T. tritici,* y la colección n° 8 de *T. levis* infectaron el centeno.

El mismo ensayo, pero en siembra tardía, proporcionó solo 2 espigas infectadas sobre 8.667 examinadas (0.2 por mil). Solo la colección n° 6 de *T. tritici,* infectó al centeno. Fué visible en este ensayo, la influencia desfavorable de la temperatura y humedad del suelo, durante la germinación del huésped, sobre la infección final.

En 1932, en el ensayo sobre especialización fisiológica, tenía 24 colecciones de *Tilletia* spp. sobre 21 huéspedes diferenciales, y el centeno de Petkus lo incluía nuevamente por concederle gran valor diferencial. En este ensayo (véase tabla n° 2) sobre 23,780 espigas de centeno examinadas, 49 aparecieron infectadas (el 2.2 por mil). Las colecciones n° 9; 11/6; 20 y 23/3 de *T. tritici* y las n° 1; 18 y 21 de *T. levis,* infectaron el centeno, resultando inmune a las 14 colecciones restantes. Las colecciones 11/6 y 23/3 de *T. tritici,* y la 10/8 de *T. levis,* son las caries recogidas sobre el centeno infectado por las colecciones n° 6; 3 y 8 en 1931. Con las colecciones 10/8; 11/6 y 23/3, además de inocular el centeno, hice lo que puede llamarse un ensayo de inoculación inverso. Es decir, inoculé las 20 variedades de trigo huéspedes diferenciales, con las caries procedentes del centeno. Los resultados obtenidos confirmaron ampliamente la identidad de las Tilletias investigadas. Como puede verse en la tabla n° 3, se obtuvo hasta 95.5 por ciento de infección en trigos inoculados con caries procedentes del centeno. Los datos consignados en la tabla n° 3, corresponden a un total de 53.534 espigas examinadas.

En 1933, en el mismo ensayo sobre especialización fisiológica, tengo en observación 43 colecciónes de *Tilletia* spp., sobre 11 huéspedes diferenciales (8 *Triticum vulgare;* 2 *Triticum durum* y 1 centeno). Hay en este ensayo aproximadamente 80.000 plantas inoculadas. Desde hace pocos días, he podido observar la aparición de espigas de centeno, infectadas por colecciones de ambas Tilletias. Es una nueva prueba de la capacidad infectante de las Tilletias del trigo sobre el centeno. Los análisis de este material (que

TABLA Nº 2.—*Resultados de la infección experimental del centeno, por 18 colecciones de T. tritici y 6 colecciones de Tilletia levis, en el año 1932–33*

Siembra: Abril 27

Especie	*Tilletia tritici*																	
Nº colección	2	3	4	5	6	7	9	11/6	12	13	14	15	16	17	19	20	23/3	24
Procedencia	L. Matienze	C. Pellegrini	La Dulce	El 21	Guatraché	Guatraché	Guatraché	Guatraché	Guatraché	Guatraché	P. Oscuro	P. Oscuro	Guatraché	Toay	Rivera	Avestruz	Guatraché	Barrow
Nº espigas	1345	1492	1277	940	1095	1158	787	771	766	733	670	906	963	1179	768	1048	976	961
Esp. infect.	—	—	—	—	—	—	4	1	—	—	—	—	—	—	—	15	1	—
(Por ciento)	—	—	—	—	—	—	0.5	0.2	—	—	—	—	—	—	—	1.6	0.1	—

	Tilletia levis							
Nº	1	8	10/8	18	21	22	Totales	Total 2 años
	Lumb	El 21	Guatr.	Guatr.	Perga.	Pico		
Nº espigas	1416	815	769	1037	1017	891	23.780	39.208
Esp. infect.	13	—	—	12	3	—	49	119
(Por ciento)	0.9	—	—	1.2	0.3	—	2.2 o/oo	3 o/oo
								39.327

TABLA Nº 3.—*Con los resultados obtenidos infectando 21 huéspedes diferenciales con Tilletias procedentes del centeno (colecciones 11/6 y 10/8) y las mismas Tilletias pero recogidas sobre trigo (colecciones 6 y 8). Año 1932*

Huésped diferencial	Huésped anterior				Total
	Centeno *T. tritici* 11/6	Trigo *T. tritici* 6	Centeno *T. levis* 10/8	Trigo *T. levis* 8	
Nº de espigas	9.677	14.161	13.008	16.688	53.534
	Por ciento	*Por ciento*	*Por ciento*	*Por ciento*	
Florence	2.4	5.1	5.8	15.2	
Kanred	19.5	3.5	93.8	90.7	
75–26–86	7.4	1.8	95.5	89.6	
Tenmarq	22.3	9.6	90.8	85.5	
Rubión	0.0	0.2	8.3	5.7	
A c d 3	1.1	0.9	2.0	1.5	
45 b	2.9	9.7	16.5	14.6	
183 c 146/25	13.3	4.4	90.5	90.5	
K–01–28	8.5	4.5	89.1	89.6	
K–0131–26	8.2	3.1	91.7	93.4	
Marquis	49.7	52.1	74.2	81.5	
Széckács 1055	1.5	2.5	61.9	49.5	
Centeno	0.2	0.0	0.0	0.0	
38 M. A.	20.6	8.8	4.6	11.3	
Oro C. I. 8220	3.3	1.0	3.1	8.3	
Kooperatorka C. I. 8861	0.0	0.0	2.5	25.0	
Wheat × Rye C. I. 8890	0.4	2.1	4.9	10.0	
Bolau Selection	0.2	0.0	0.9	10.3	
Grand Nº 4 Selection	0.0	0.2	4.2	13.0	
Ioturk (Iowa Nº 1184)	—	2.0	9.2	38.0	
Minturki × (Beloglina × Buffum) C. I. 8033	—	9.3	3.0	13.6	
Promedios	8.5	5.7	35.8	39.8	

como es lógico deben postergarse hasta la maduración del mismo) arrojarán nuevas luces sobre este interesante caso de parasitismo.

ALGUNAS CONSIDERACIONES SOBRE LOS RESULTADOS

a. *La Especialización Fisiológica y la Resistencia Genérica.*—Examinando la tabla nº 4, resulta evidente que solo ciertas formas fisiológicas especializadas, son capaces de atacar el centeno. Quizás para los técnicos no familiarizados con estos conceptos, sea necesario aclararlos. Las f.f. esp., biotipos o razas, de un hongo, son variaciones de una misma especie, que conservando las características sistemáticas específicas, se singularizan sin embargo por otros caracteres subespecíficos. En realidad ocurre con ciertos hongos, que tienen un polimorfismo tan marcado como el que nos es habitual

TABLA Nº 4.—*Con las 3 formas fisiológicas de T. tritici y 4 f.f. de T. levis que atacaron al Secale cereale v. vulgare, en los años 1931 y 1932*

Especie del hongo	*T. tritici*						*T. levis*			
Colección Nº	3	6	9	11/6	20	23/3	1	8	18	21
a) Forma fisiológica	II	V	V	V	IX	II	I	II	III	IV
Procedencia	C. Pellegrini	Guatraché	Guatraché	Guatraché	Avestruz	C. Pellegrini	Lumb	El 21	Guatraché	Pergamino
Por ciento infec. 1931	1.7	1.1	2.4	.....	.....	.....	0	2.9	.....	.....
" " " 1932	0	0	0.5	0.2	1.6	0.1	0.9	0	1.2	0.3

a) Las formas fisiológicas que se presentan en este cuadro, han sido aisladas por el autor en sus ensayos sobre especialización fisiológica de las caries argentinas del trigo. En 1931, sobre 6 plantas infectadas, solo una estaba totalmente infectada y tenía 29 espigas, (levis 8, f.f. II). Reducción talla 11 %.
Con tritici 6 (f.f. V), hubo reducción longitud espigas 18%.

observar en fanerógamas superiores. En *Puccinia graminis* por ejemplo, se han establecido ya, 130 f.f. esp. y su número tiende a aumentar incesantemente.

Hay distintos medios de diferenciar las f.f. de un hongo; según Stakman (23) habría 4 métodos generales de diferenciación:

1º) por su comportamiento patogénico sobre ciertos huéspedes diferenciales, (el más antiguo).
2º) por sus características culturales.
3º) por sus relaciones físico-químicas y
4º) por su morfología, (método limitado).

Desde el descubrimiento de Schroeter (22, pp. 69–70), hasta 1918, se hize labor clásica sobre especialización fisiológica de las Puccinias y del *Erisiphe graminis,* pero solo recientemente Kniep (13), tuvo la primer evidencia de la especialización de los carbunclos de los cereales (Ustilagáceas). Respecto a las Tilletias del trigo, el primero en demostrar su especialización fué Reed (17), aunque ya Faris (5) había obtenido algunos datos sugestivos. Trabajos posteriores de Sampson (21), Gaines (7), Rodenhiser y Stakman (19), Reed (18), Roemer (20), Holton (10) y recientemente Bressman (2) han confirmado de un modo indubitable los primeros hallazgos de Faris y Reed. Todos estos autores llaman formas fisiológicas en las *Tilletia* spp. a las colecciones de clamidósporos de determinada procedencia, que se singularizan por su comportamiento patogénico sobre ciertos huéspedes diferenciales. La conducta patogénica, ha ofrecido

casi siempre una regularidad remarcable, no obstante tratarse dichas colecciones de verdaderas "poblaciones."

En otros hongos las f.f. se han establecido sobre descendencias unisporidiales. En las Tilletias ciertos detalles de técnica, han hecho hasta ahora imposible adoptar el mismo criterio. (Una descendencia unisporidial, es equivalente a una "línea pura" o "pequeña especie" en el sentido de Johannsen). Es probable que en un futuro próximo, salvados dichos inconvenientes, podrá adoptarse un criterio más próximo al de las descendencias unisporidiales.

El polimorfismo de *Tilletia* sp., aparece muy marcado, y a mi juicio solo es cuestión de tiempo y trabajo establecer mas de un centenar de formas como en *Puccinia graminis*. Bressman (2), trabajando con 48 colecciones de *T. tritici* y 46 de *T. levis*, de procedencia muy variada, señala en 1931, 6 f.f. en *T. levis* y 4 en *T. tritici*.

Los otros autores citados han encontrado otras formas, pero es difícil establecer la interrelación que puede existir entre las formas de los diversos autores, porque no se han usado los mismos huéspedes diferenciales. Por otra parte, hay detalles de técnica que se oponen al uso de una serie "standard" de huéspedes en cualquier lugar geográfico, pues entre otras cosas, aún usando la misma serie, los resultados obtenidos en dos puntos geográficos son incomparables, por la influencia del medio. Como se vé, las Tilletias ofrecen complicaciones, que no valen para las Puccinias. Los autores citados, han establecido la especialización de las Tilletias, usando preferentemente el método nº 1. Alguno como Bressman, tambien ha utilizado el método nº 3. Casi todos han hecho observaciones sobre variación morfológica (método 4). Ninguno ha podido usar el método nº 2. Yo he utilizado los tres métodos viables.

En la tabla nº 5, puede verse que establezco 9 f.f. en *Tilletia tritici* y 4 f.f. en *Tilletia levis*, todas argentinas, y difundidas en el Sud de la República. En mi trabajo de 1933 aún no publicado, me ocupo con cierta extensión de esta investigación.—La severidad del método experimental, y el

TABLA Nº 5.—*Con las formas fisiológicas de T. levis y Tritici argentinas, y las colecciones de carie, que han permitido establecerlas*

Formas fisiológicas	*Tilletia levis*				*Tilletia tritici*								
	I	II	III	IV	I	II	III	IV	V	VI	VII	VIII	IX
Colecciones Nº	1	8	18	21	4	3	2	5	6	16	17	19	20
		10	22?		12	23		13	7				24?
								14	9				
								15	11				

TABLA Nº 6.—*Mostrando el polimorfismo de las caries argentinas en solo el departamento pampeano de Guatraché*

Formas fisiológicas	*Tilletia levis*		*Tilletia tritici*			
	II	III	IV	V	VI	IX
Colecciones Nº	8; 10	18	5; 13	6; 7 9; 11	16	20

volumen del material analizado, defienden elocuentemente mis conclusiones. Solo en los ensayos de 1931 y 1932, se observaron 632,252 espigas. En un futuro próximo me será posible diferenciar mas formas. No debe olvidarse que en tal sentido hay posibilidades ilimitadas. No solo juegan su rol las mutaciones, sino que se forman nuevas combinaciones por hibridación. Asi lo demuestra Flor (6) en un trabajo reciente, en que se prueba el heterotalismo de las Tilletias, hecho supuesto, pero que dificultades de laboratorio, no habían podido evidenciar hasta ese momento.

b. *Características de la Infeccion sobre el Centeno y Variaciones Morfológicas de Orígen Patológico, del Huésped.*—Resulta evidente que el centeno, ofrece una considerable resistencia a los ataques de *Tilletia* sp. No solo es immune a gran número de colecciones (y f.f. esp.), sino que las f.f. esp. que lo atacan solo pueden infectar muy pocas espigas. Bressman ha podido obtener hasta un máximo de 6.1 por ciento de infección, con la f.f. 6 de *T. tritici,* sobre la variedad de centeno Oregon Sel. Nº 1. En mis ensayos, la infección máxima fué obtenida con la forma fisiológica II de *T. levis* (Colección 8, procedencia "El 21"), en la siembra temprana del año 1931, y alcanzó solo a 2.9 por ciento. Resulta sugerente, que sobre 9 f.f. que establezco en *T. tritici,* solo 3 f.f. atacan el centero, mientras que sobre 4 f.f. que establezco en *T. levis,* todas lo atacaron. Por otra parte el material era mas abundante respecto a *T. tritici,* pues tenía 18 colecciones de esta especie y solo 6 de *T. levis.* Esta mayor patogenicidad de la *Tilletia levis* sobre el centeno, vuelve a fijar mi atención sobre la hipótesis de Bressman (1), quien al obtener el resultado que conocemos imaginó "si la *T. secalis* (Corda) Kühn, no sería una f.f. de *T. tritici,* y debía ser considerada como tal." Esta hipótesis se basa en la virulencia que observa en su f.f. nº 9, y el conocimiento, que los esporos de *T. secalis* son reticulados como los de *T. tritici.*

Basándonos en la virulencia, podemos suponer ahora: como no se ha establecido *T. secalis levis,* (es decir a clamidósporos lisos)?. Quizás no sea mas que una falla de la observación, su supuesta inexistencia. La hipótesis de Bressman es digna de concederle alguna atención experimental. No creo que se haya establecido la especificidad de *T. secalis,* y ensayado su

capacidad patógena sobre los trigos. Será necesario hacer una atenta experimentación, para aclarar definitivamente este asunto.

Volviendo a las características de la susceptibilidad en el centeno, tenemos que sobre 6 plantas infectadas en 1931, solo 1 estaba totalmente infectada, (véase tabla N° 7).

TABLA N° 7.—*Con detalles sobre las plantas de centeno infectadas en la siembra temprana de 1931*

Tilletia	Plantas			Espigas[a]		Inf. ind.
	Sanas	infectadas		Sanas	infectadas	
		Parcialmente	Totalmente			
						Por ciento
tritici colec. 3	19	1	–	36	15	29.3
" " 6	20	1	–	26	8	23.6
levis colec. 8	26	–	1	–	26	100.0
tritici colec. 9	12	2	–	112	15	11.9
tritici colec. 6[b]	39	1	–	13	2	13.4
Totales	116	5	1	187	66	
Porcentajes		4.1	0.9		26.1	

[a] El número de espigas corresponde unicamente a las plantas infectadas.
[b] Corresponde a la siembra tardía de 1931.

En 1931, tuvimos un "stand" (número de plantas por m^2.) muy reducido, por ser la semilla de centeno de algunos años atrás. Pero el macollaje (innovaciones) fué extraordinario. En 1932, tuvimos un "stand" normal, pero el recuento del material se hizo unicamente sobre las espigas. Puede observarse que sobre 122 plantas de centeno, en las parcelas atacadas, se encontró 4.1 por ciento de plantas parcialmente atacadas y sólo 0.9 por ciento totalmente atacadas. Los porcentajes de "infección individual" oscilaron entre 11.9 y 100 por ciento.

Es apreciable cierto "nanismo," es decir reducción de la longitud de la espiga. Las mediciones realizadas acusan un 18 por ciento de reducción. Por otra parte la planta totalmente infectada, por *T. levis* colec. 8, comparada con el promedio de tallas de plantas sanas del mismo lote, tambien ofreció "nanismo" aunque poco acentuado (11 por ciento). Bressman por su parte dice que no pudo verificar "nanismo" en su material. El número de "granos de caries," encontrado en las espigas infectadas ofrece un detalle de interés. En esta variedad de centeno, se encuentra una considerable "lacunosidad" en las espigas, resultando de que muchas flores no

producen semilla, por azares de la fecundación cruzada (alogamia) que es común al género Secale.

La "esterilidad parcial" de las espigas sanas contrasta con la "fertilidad total" de las espigas infectadas, en las cuales todas las flores se han convertido en granos de caries (smut balls). He verificado microscopicamento la naturaleza de los clamidósporos de ambas Tilletias y en cada caso, les ejemplares recogidos sobre centeno eran típicos. Por otra parte su tacto es untuoso y posee la característica fetidez propia de las colecciones originarias. Quizás convenga hacer presente que no todas las caries del trigo, exhalan el hedor fétido, que ha servido para caracterizar su presencia con el nombre de "carbón hediondo," "stinking smut," etc. Recientemente ha sido demostrado por bioquímicos canadienses, que dicho olor se debía a la presencia de trimetilamina en los clamidósporos. Hanna (8) cruzando Tilletias con y sin trimetilamina, probó que la presencia de la trimetilamina era un caracter transmisible por herencia que se debía a factores dominantes. Igualmente dominante es el reticulado de los clamidósporos.

SUMMARY

Experimental infection of rye (var. Petkus) by wheat bunt: *Tilletia tritici* (Bjerk.) Wint. and *T. levis* Kuehn.

During the years 1931–33, the author succeeded in infecting experimentally the rye variety known as Petkus with certain physiological strains of *Tilletia tritici* and *T. levis*. Preliminary studies indicated that the rare *Tilletia secalis* does not occur in Argentina. A survey of the literature yields reports of the occurrence of wheat Tilletias to a very limited extent on rye in Washington, Oregon, and Kansas.

In the experimental work in question seed grain was dusted shortly before planting time with the spores of the particular smut collection being studied. Grains (seeds) were planted separately 7.5×20 centimeters or at times 15×15 centimeters, with 180 plants to the unit plot. In 1931–32, 6 collections of *T. tritici* and 2 of *T. levis* were used, two sowings being made, April 23 and July 20. From the first planting, 68 infected spikelets were found in a total of 6,761, approximately 1 per cent; from the second planting, 2 only were infected in a total of 8,667, or .02 per cent. Three collections of *T. tritici* and one of *T. levis* produced infection.

In 1932–33, 18 collections of *T. tritici* and 6 of *T. levis* were used, plantings being made on April 27. Forty-nine infected spikelets were found in a total of 23,780 examined, or .22 per cent. Average infection for the two years was .3 per cent, or 119 infected spikelets out of a total examined 39,208. Of the smut collections used, 4 of *T. tritici* and 3 of *T. levis* produced infection.

During 1933–34, 43 collections of Tilletia were used, all collected in Argentina by the author and his colleagues with the exception of one received from Germany. Preliminary results of this series, which included 11 different hosts, indicate further positive results, but definite figures await maturity of the crop.

In connection with these infection studies, the author has established by the differential host method 9 physiological forms of *T. tritici* and 4 of *T. levis*. Of the forms of *T. tritici*, only 3 were found to infect rye.

ESTACIÓN EXPERIMENTAL DE GUATRACHÉ.
DIRECCIÓN GENERAL DE AGRICULTURA.
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NOMENCLATURE OF THE TOBACCO DOWNY MILDEW FUNGUS

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For many years the organism causing the tobacco downy mildew or blue mold was called *Peronospora hyoscyami* deBary. Recently the authors, as well as various other workers, have recognized that this name is incorrect because the tobacco downy-mildew fungus does not infect *Hyoscyamus niger*. The question has been raised as to whether this fungus should be given a new name, or should be referred to some other species already described. This question has been decided both ways in recent publications by Adam (1) and by Wolf (7), but in each case the conclusions were drawn without the author's having had access to the type material of *P. nicotianae* Speg., as collected by Spegazzini. The writers have been able to secure such type material from the Spegazzini herbarium.[1] Also it has been assumed that the only available description of the Spegazzini species was a very brief one published in 1891 (4) and listed in Saccardo (3). It has been discovered that a later publication was issued in Spanish in 1898 (5) and republished in Portuguese (6). This gives a very full account of both fungus and disease. It is proposed to consider the question of nomenclature in the light of the more complete information now available.

The Spegazzini herbarium material consisted of 4 collections, dated 1888, 1896, 1897, and 1899. The 1896 collection showed conidiophores and conidia and the other 3 showed oospores. The material was cleared in lactic acid and mounted in lacto-phenol for study.

Adam (1) lists 5 points of difference between *Peronospora nicotianae* and the Australian organism for which he proposes the name *P. tabacina*. Considering these points in detail, he states, "(1) The ultimate branchlets of *P. nicotianae* are uniformly longer and more acutely pointed than are those of *P. tabacina*." His measurements are from 8 μ, or less, to 14 μ. Average measurements[2] from collections made in the United States ranged from 8.35 μ to 11.6 μ. Similar averages for the 1896 Spegazzini collections were 9.72 μ to 13.34 μ. The difference, if it exists, is not great. Adam (2)

[1] The writers wish to express their deep appreciation of Dr. Juan B. Marchionatto, Chief of the Laboratory of Phytopathology of the Ministry of Agriculture, Buenos Aires, Argentina, for courtesies extended and to Dr. Maria M. Job, Museum of the University of La Plata, Argentina, for her kindness in lending specimens from the Spegazzini herbarium without which this paper could not have been prepared.

[2] These measurements were determined as follows: Ten measurements were made in a given microscopic field and averaged; from 5 to 10 sets of readings were made for each factor studied and the final figures given in this paper represent the range of the 5-to-10 averages thus obtained.

finds that, "The conidia of *P. nicotianae* (19 μ × 10 μ) are smaller than those of *P. tabacina* (22 μ × 17 μ)." Here the greatest difference is in breadth. The average breadths of United States collections ranged from 12.45 μ to 16.46 μ. The average of the Spegazzini conidia was from 16.72 to 17.44 μ (Fig. 1). Thus, instead of being smaller, the writers actually

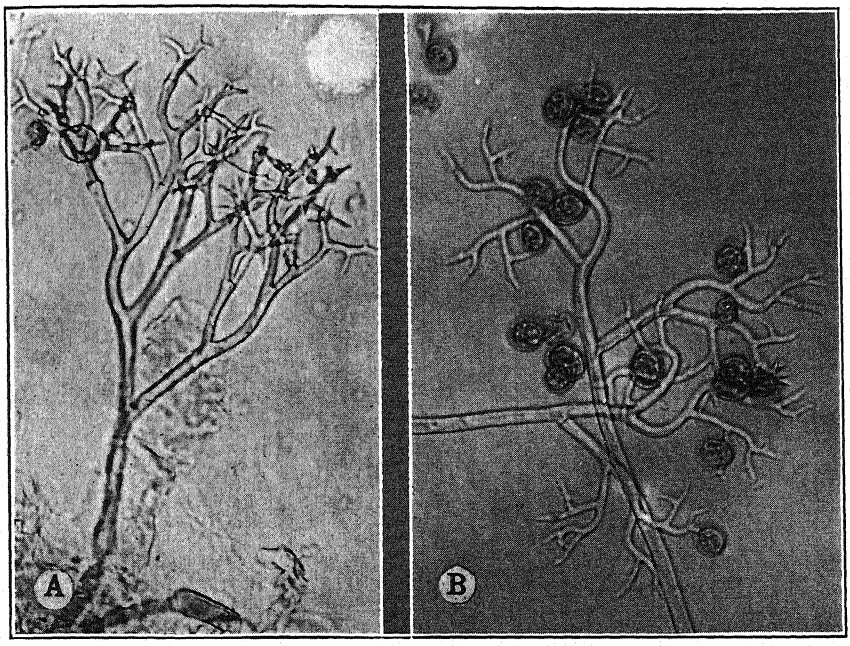


FIG. 1. A. Mature conidiophore of *Peronospora nicotianae* Speg. from material collected by Spegazzini, 1896. × 300. B. Mature conidiophores of *Peronospora tabacina* Adam from material collected in Maryland, 1933. × 300.

found the Spegazzini conidia larger. However, Angell and Hill (2) have shown that size of both conidia and conidiophores is subject to great variation under different conditions; hence it is concluded that the differences cited by Adam are without significance.

Concerning oospores, Adam points to the following: "(3) The diameter of the oospores of *Peronospora nicotianae* (50–70 μ) is much greater than that of *P. tabacina* (35–60 μ, average 46 μ)." Oospore material collected in the United States gave average measurements ranging from 32–40 μ. The Spegazzini collections gave similar averages from 62 to 78 μ. There was no question but that the Spegazzini oospores were much larger. Regarding color of oospores Adam states, "(4) The color of the oospores of *P. nicotianae,* is golden brown, that of *P. tabacina* dark brown." This color difference was found to be very striking and as stated. The marked differ-

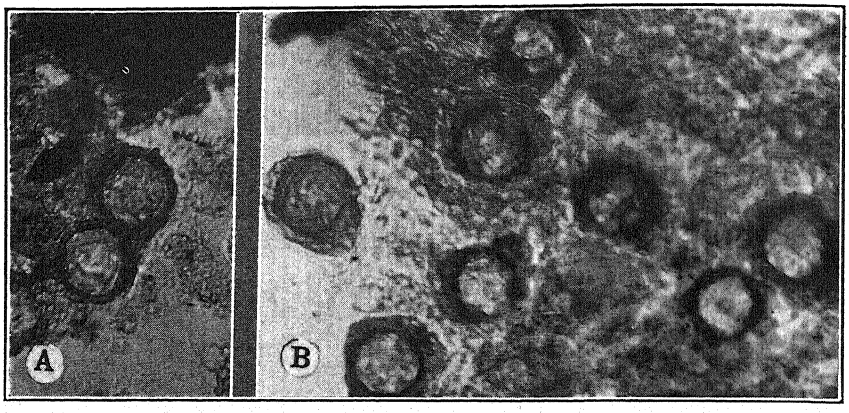


FIG. 2. Oospores of *Peronospora tabacina* Adam. ×300. A. From material supplied by F. Wolf. B. From material collected at Oxford, N. C., 1934.

ence in wall pattern also is noted by Adam. Figures 2 and 3 show the comparative size of the oospores of the two species and illustrate the characteristic wall pattern of *P. nicotianae*. It should be noted here, however, that Spegazzini did not germinate his oospores and produce the disease, nor has this been done with the oospores that the present writers and others have associated with our tobacco mildew fungus.

The grounds advanced by Wolf for believing our fungus and *Peronospora nicotianae* to be identical are now considered. He points out that the differences in size of conidia and conidiophores have no great significance. Regarding size of oospores he notes that "the size recorded by Spegazzini is 50 to 80 μ and ours 45 to 75 μ" but makes no comment as to color or wall pattern. This size, 45 to 75 μ, approaches the Spegazzini measurements closely, but collections of the present writers in the United States do not show such large oospores. The oospores on a slide kindly supplied by Dr. Wolf averaged 39.49 μ and were of the usual type.

The extensive account left by Spegazzini (5) represented a study that continued from 1888 to 1898, and his first description of the species, published in 1891, is both modified and greatly extended in this later publication. He notes that, "the disease appears first in the form of small, more or less rounded spots that are visible to the naked eye, even at a distance. These spots enlarge irregularly and by degrees until they invade not only one, but all of the leaves of the plant extending in many cases as far as the stalk; they are yellowish, more or less pronounced, and very plainly visible; the portion of the plant invaded shows a very slight increase in thickness, at the same time becoming brittle to the point of easily breaking if bent. In addition, the leaves are seen to be slightly convex, the convexity being turned toward the upper part; the stems in the diseased plants also are

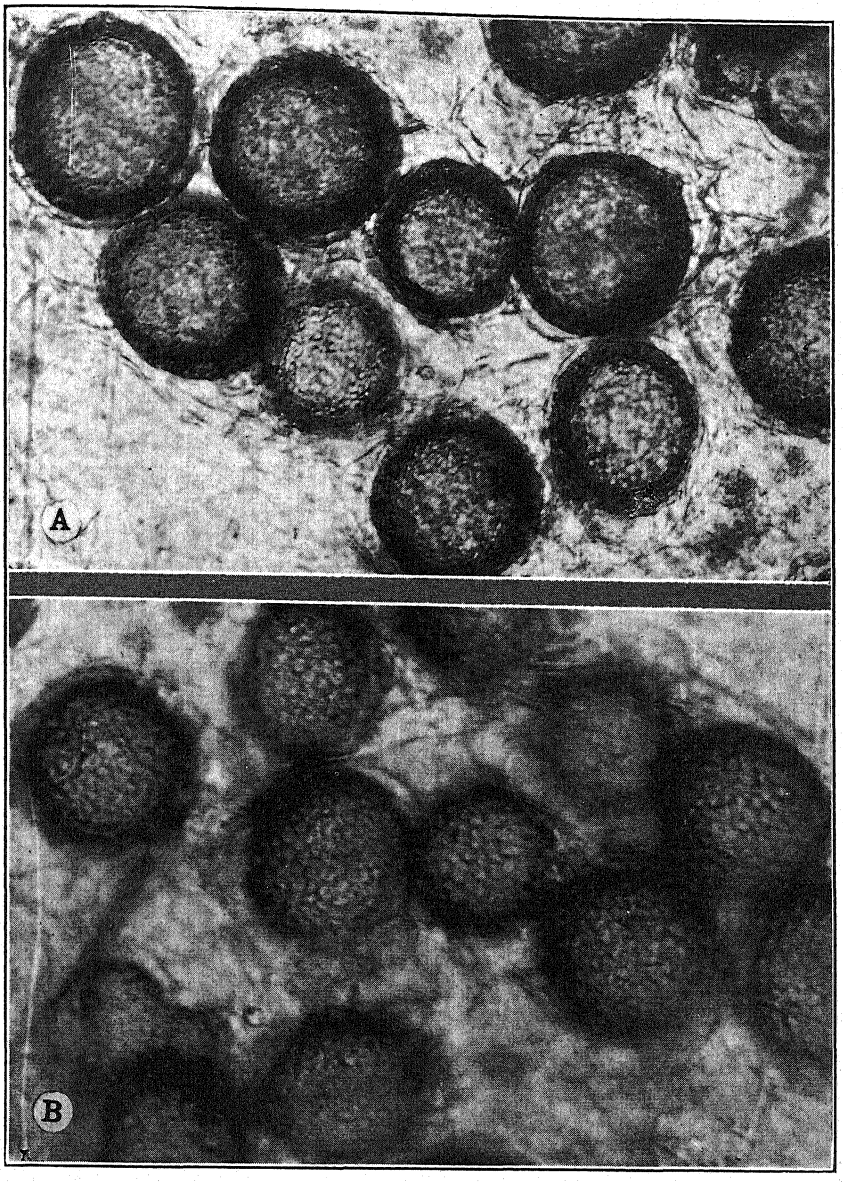


FIG. 3. Oospores of *Peronospora nicotianae* Speg. from material collected by Spegazzini, 1897. ×300. A. Focused to show size and envelope. B. Focused to show pattern of epispore.

yellow, succulent, and brittle, and have a great tendency to fasciation; it is not uncommon to see branches that, instead of following a straight line, are shaped more or less like a fish hook. The infection always begins with the lower leaves, especially with those lying on the ground and gradually extends to the stalk and upper leaves." He also mentions the downy growth of conidiophores on the lower leaf surface. Regarding conidiophores and conidia, it is stated that, "These branches are erect and straight, rising ½ to 1½ mm. in height, are almost cylindrical and have a thickness at their base almost three times that of the internal hyphae from which they are derived, measuring 10–12 microns in diameter, while the diameter of the upper part is less. The conidia or reproductive bodies produced at the ends of these branches are elliptical or slightly oval in shape, obtuse at both ends, and perfectly smooth. They sometimes have a very small tubercle at the lower end where they are attached to the fruiting branches. Their size is variable, measuring 15–25 μ in length and 8–14 μ in width. . . . The protoplasm with which they are filled is always colorless and consists of a very large number of granules each of which changes into a zoospore when the conidia are kept some time in water." Concerning oospores he says, "These oospores develop within the parenchyma cells themselves, from which they completely absorb the nutrient substance, provoking at the same time hypertrophy of the cell wall. A kind of chitinized covering or colorless capsule is formed. This is globular, or more or less obtusely angular, 80–100 μ in diameter, with walls having a thickness varying from 10–15 μ. It is rigid and completely smooth. On the inside of each cell there is one globular oospore, 50–80 μ in diameter, at first of a yellowish gray color, later becoming reddish gray and more or less dark or opaque owing to the thick wall of the chitinized epispore which shows a well defined design, very similar to a bee's honey comb."

"This species very closely resembles *Peronospora hyoscyami* of de Bary with which, however, I do not identify it because it lives on a different plant and because the oospores of the latter are not known." As to pathogenicity, Spegazzini reports that he collected seed of available wild species of Nicotiana, as well as cultivated varieties, and all were severely attacked by the disease. Before coming to a conclusion as to whether this mildew described by Spegazzini is the same as the disease that occurs in the United States and Australia attempts were made to obtain fresh material from the Argentine. We were advised, however, that this disease has not been observed there for at least the past 10 years.

Considering the statements in the Spegazzini publication, 2 points indicate that *Peronospora nicotianae* and our fungus may be the same. Both attack numerous species of Nicotiana and it seems unlikely that there should be two Peronosporas of such similar pathogenicity. Also, Spegazzini noted

a strong resemblance between his species and *P. hyoscyami,* and our tobacco downy-mildew fungus also resembles *P. hyoscyami.* However, in light of the fact that comparatively few species of Peronospora were known at that time, and that most conidial stages in this genus resemble each other, no importance can be attached to this observation. There is very little additional positive evidence. The description of disease symptoms does not fit particularly with those we are accustomed to seeing, except in a general way as would be true of any downy mildew. The description of oospores emphasizes again that the oospores associated with *P. nicotianae* are very different from those of our tobacco mildew. There are no significant differences as to general characters of conidiophores and conidia, but Spegazzini states that his conidia germinated indirectly with the formation of zoospores. Since at the time this was written he had had the organism under study for years, and the conidia germinate readily in water drops, it seems likely that he would have observed this germination many times. The conidia of our mildew fungus have been germinated under a wide variety of conditions and have never been observed to form zoospores. Consequently, use of the name *P. nicotianae* would necessitate the assumption that Spegazzini was mistaken in his description of both the oospore and conidial stages. While this is possible, it is possible also that a fungus fitting his description exists in South America, more especially since the writers have obtained no positive evidence that tobacco growers there have been troubled by a mildew in recent years, or that the disease described by Spegazzini was ever a crop problem.

In view of this uncertainty, it would appear inadvisable to apply the name *Peronospora nicotianae* to our fungus. *P. tabacina,* as described by Adam, on the other hand, fits our organism in all essential particulars; hence, the use of this name is recommended.

BUREAU OF PLANT INDUSTRY.

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3. SACCARDO, P. A. Sylloge Fungorum 11: 243. 1895.
4. SPEGAZZINI, C. Phycomyceteae Argentinae. Rev. Argentina Hist. Nat. 1: 36–37. 1891. (Reprint pp. 11–12. 1891).
5. ————. Sobre una neuva enfermedad del tobaco y el polvillo de la alfalfa. Oficina Quimico-Agric. Prov. Buenos Aires Bol. 4. 1898.
6. ————. Sobre una nova molestia do fumo. A Lavoura 3: 18–20, 74–79. 1899.
7. WOLF, F. A., L. F. DIXON, RUTH MCLEAN, and F. R. DARKIS. Downy mildew of tobacco. Phytopath. 24: 337–363. 1934.

PHYTOPATHOLOGICAL NOTE

Comments on the Article[1] *by A. Kozlowski on "Little Leaf or Rosette of Fruit Trees in California."*—The recent article by Dr. Kozlowski in this journal, while not a contribution from the University of California, and not designated as such, may confuse some readers of previous reports on the little-leaf disease in California, by Chandler, Hoagland, and Hibbard. We therefore desire to offer the following comments:

Dr. Kozlowski was formerly employed by this institution, in a temporary capacity, to assist in certain bacteriological studies on soils producing the little-leaf disease. On his own initiative Dr. Kozlowski has undertaken to interpret certain phenomena of soil science and plant pathology which fall outside his special field of knowledge. The conclusions reached are founded on a misinterpretation of certain limited observations made in the course of the general investigation and are irrelevant or inconsistent with well established facts. Dr. Kozlowski omits all reference to the most fundamental aspect of the experiments on little leaf; namely, the specific function of zinc, which clearly is not merely that of a fungicide. The views of Dr. Kozlowski should not be considered as in any way related to our own. —W. H. CHANDLER, D. R. HOAGLAND.

[1] Kozlowski, A. Little leaf or rosette of fruit trees in California. Phytopath. 25: 275–278. 1935.

BOOK REVIEWS

Root Rots and Degeneration of the Strawberry

Several articles on strawberry diseases in England and Canada have appeared recently. Papers[1] by Akenhead, Harris, Berkeley, and Massee review "degeneration" in England, while Hildebrand[2] and Truscott,[3] following work by Walker and Berkeley, report on extensive studies of root rots in Canada. The complexity of the root-rot problem was indicated by Walker when he isolated 39 different organisms from diseased roots and crowns in 1927 and considered *Ramularia* sp. and *Fusarium* sp. as important causal organisms, the former being the more serious. Hildebrand, in his recent paper, reports isolating 20 different genera from 125 healthy-appearing young roots. Truscott lists as primary parasites species of Pythium, Fusarium, Alternaria, Ramularia, Rhizoctonia, Verticillium, Cylindrocladium, and Asterocystis, and notes the frequent occurrence of both a Plasmodiophoraceous fungus and a Phycomycetous mycorrhizal fungus. Hildebrand noted the almost universal presence of 2 so-called endotrophic mycorrhizal fungi, one of which was a Rhizoctonia type.

In the English articles Berkeley simply summarizes the literature on strawberry root diseases throughout the world and concludes that root rot is wide-spread and important and that, although the severity of root rot may be increased by several conditions, it may be the direct result of fungus attack and may occur in any type of soil and in plantations growing under the best cultural conditions. He already has found a *Coniothyrium* sp. associated with root rot in England.

The causal agents of root rot, as recorded in the literature noted by Berkeley, are Rhizoctonia in Michigan, Washington, New York, Oregon, Utah, Australia, and France; *Leptosphaeria coniothyrium* and *Pezizella lythri* in Michigan; *Ramularia* sp. in Canada; *Fusarium* sp. in Mississippi; *Pythium* sp. in Australia, France, Scotland, and Louisiana; *Diplodina* sp. in England, a sterile fungus in the Southern States; and a number of the Fungi Imperfecti in Scotland. He notes also Verticillium wilt in California; the Lanarkshire disease caused by an endotrophic mycorrhiza in

[1] Akenhead, D., R. V. Harris, G. H. Berkeley, and A. M. Massee. The "degeneration" of the strawberry. I.—The pomological aspect. II.—Virus. III.—Root rot. IV.—Insects and other animals. Imp. Bur. Fruit Production, East Malling, Eng., Tech. Commun. 5. 1934.

[2] Hildebrand, A. A. Recent observations on strawberry root rot in the Niagara Peninsula. Canad. Jour. Res. 11: 18–31. 1934.

[3] Truscott, J. H. L. Fungous root rots of the strawberry. Canad. Jour. Res. 11: 1–17. 1934.

England; a crown rot caused by *Colletrotrichum fragariae* in England; a root rot in Florida caused by *Sclerotium rolfsii;* and 3 bacterial organisms producing decay of roots in Florida.

Such an array of root parasites is enough to make both the strawberry grower and strawberry runner squirm! Even the relatively definite work by Zeller, by Thomas, and by Strong and Strong in sections of the United States where a single organism, or at most 2 organisms were the important ones, leaves many unsettled problems. Control measures recommended are *setting clean plants in clean soil,* but practical directions for obeying those words are a long, long way off. Possibly conditions in Canada and England are worse, or rather more complicated than in some sections, but we suspect they are not more so than in other sections of the United States.

"Degeneration" of the strawberry in England was considered by Berkeley in relation to root rots; but, by Akenhead, in relation to the importance of vigorous runner plants, the importance of early planting, and mixtures of varieties that are apparently much more common in England than in the United States. Degeneration was considered by Harris in relation to strawberry viroses, one of which seems to be wide-spread in England. He states that experiments are in progress to determine the vectors of the "yellow-edge" virus, to study strain and varietal susceptibility, and to originate virus-resistant varieties. He also states that evidence has been obtained that certain of the clone races are more susceptible than others to outside infection.

Massee reviewed the work on the bud nema and considered that there is ample evidence that the eelworm factor may be important. In reviewing the work on aphids he states that many plantations degenerate even though aphids do not occur in them. The strawberry tarsonemid mite is found in most parts of the British Isles, and differences in varietal susceptibility were noted. This mite is being tested as a vector of the yellow-edge virus. In the 4 papers on degeneration of the strawberry, 161 references are listed. Hildebrand lists 13 and Truscott 25 papers.

Comparing conditions in England with those in the United States, Central California and the Pacific Northwest have virus problems as serious as those in England. However, more is known of the virus troubles on the Pacific Coast than of those in England. In the Eastern States the crinkle virus of the Northwest has been seen several times but is apparently not wide-spread. An apparent virus was observed by the reviewer in New England and in Maryland in 1934 in a few fields. Either the yellow-edge and "xanthosis" viroses are absent in the Eastern States or the varieties grown are resistant to them or mask their effects. Certainly through the past decades there has been opportunity for their introduction from Europe and the Pacific Coast. Possibly eastern varieties, being somewhat resistant,

do not have marked symptoms but slowly succumb. It seems hardly possible that root rot is so serious in the United States on the whole as it is in England and in Ontario, Canada. Resistant varieties, more suitable soils, a more southern latitude and climate, and recognition of root injury due to winter injury (Roberts), to ammonium sulphate (Lineberry), and to root aphis may be contributing factors. Certainly we may expect a stimulus to research on strawberry diseases in this country following these excellent papers.

Another paper by Berkeley and Lander-Thomson,[4] received after the above was written, reports proof of the pathogenicity and also the identification of 5 species of soil fungi, namely, *Coniothyrium fuckelii* Sacc., *Hainesia lythri* (Desm.) v. Höhn., *Cylindrocarpon radicicola* Wr., *Fusarium orthoceras* App. and Wr., and *Pachybasium candidum* Sacc. Of the 5, *Fusarium orthoceras* and *Coniothyrium fuckelii* appeared most virulent and the most important parasites, although *Pachybasium candidum,* a less common fungus, was very virulent. The symptoms of all five were said to be similar in all respects to those given by Strong and Strong for *Coniothyrium fuckelii* in Michigan. The *Ramularia* sp. reported by Walker was considered identical with the *Cylindrocarpon radicicola* Wr. The Cylindrocarpon and Fusarium fungi have been found on raspberry roots as well. They conclude that root rot may be due to any or all 5 fungi. However, there seemed to be seasonal and regional distribution of the different root rots. This paper adds much to our understanding of these very difficult troubles and also indicates how difficult (and important) adequate research on root rots may be.—GEORGE M. DARROW, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, Washington, D. C.

Paul Sorauer. Handbuch der Pflanzenkrankheiten. Erster Band. Die Nichtparasitären und Virus-Krankheiten. Zweiter Teil. Sechste, neubearbeitete Auflage. Herausgegeben von Dr. O. Appel. Pp. VIII × 553. Paul Parey, Berlin. 1934. 46.00 Gold Marks.

Volume I of the sixth edition of Sorauer's Manual deals with non-parasitic and virus diseases. It is issued in two parts of approximately 500 pages each. The first part was published a little less than a year ago and was reviewed in volume 24 (p. 427–430) of this journal; the second part appeared more recently and will be discussed in the succeeding pages.

The book is in every respect a worthy companion to the introductory volume and the subject matter climaxes in a most interesting account on virus diseases. There are six main divisions:

[4] Berkeley, G. H., and I. Lander-Thomson. Root rots of strawberry in Britain. The "black lesion" type of strawberry root rot. Jour. Pom. & Hort. Sci. 12: 222–246. 1934.

Chapter 4. Diseases conditioned by internal causes. P. 1–80.
" 5. Unfavorable soil condition as cause of disease. P. 80–168.
" 6. Wounds. P. 168–240.
" 7. Smoke injuries. P. 243–310.
" 8. Injuries caused by factory wastes and sewage. P. 310–329.
" 9. Virus diseases. P. 329–512.

The chapter on plant diseases conditioned by internal factors is divided into developmental and functional disturbances during early embryogeny, the vegetative, and the reproductive period. The viewpoint of treatment is that of the geneticist. Developmental disturbances during the vegetative period deal primarily with the genetic setup responsible for dwarfing, atrophy, or loss of organs, chlorophyll defects, and necrosis. Disturbances during the reproductive period are listed as: failure of flower development, premature abscission of flowers, abortion of floral parts, notably pistils and stamens.

Chapter 5, on unfavorable soil conditions as cause of disease, comprises approximately 90 pages. It deals with both the physical and the chemical aspect of such conditions. Unfavorable physical soil states are conditioned by abnormal soil structure and an unbalanced water, air, and temperature economy. Soil loses its friability primarily through losses of electrolytes and soil colloids. A prerequisite for such a change is a humid climate; on the other hand, in arid regions with predominately unilateral water movement, there may be even an accumulation of salts in the top layers instead of a leaching. A sufficient water supply is in most cases the deciding factor for normal growth. Most growth disturbances are caused directly or indirectly by an abnormal shift in the water balance of the soil. Water deficiency manifests itself first of all in wilting, but it may produce disease symptoms such as white-spike disease of grasses, crown gall of beets, stippin apples, etc.

Unfavorable chemical changes greatly affect plant nutrition, soil reaction and are largely responsible for the accumulation of harmful substances. Numerous examples might be cited, *i.e.*, boron deficiency, responsible for heart rot of beets, and magnesium deficiency for "sand drown" of tobacco and corn. The subject of Bodenmüdigkeit—soil sickness—is treated in great detail and the various hypotheses set forth in explanation are critically examined.

The chapter on wounds is treated from the following aspects:

1. Cause of wounding: precipitation in form of rain, snow, and hail, wind, lightning, fire, excessive heat or cold and injuries caused by animal and man.

2. Reaction of plants to wounding: functional disturbances, wound heal-

ing, wound hormones; formation of wound gum and tyloses; genesis of wound cork, callus and gnarls in wood.

3. Cell and tissue regeneration following wounding; loss of roots and shoots and their restoration in woody and herbaceous plants.

4. The effect of wounding on the general development of plants.

Chapter 7 on smoke injuries deals with the toxic effect of certain chemicals such as sulphurous and sulphuric acid, chlorine, hydrochloric acid, illuminating gas, nitric acid, ammonia, asphalt fumes, etc., on the normal development of plants. The effect may be either acute or chronic and then usually invisible, manifesting itself in a gradually lowered performance. Chronic injuries, if prolonged, may become acute and then diagnosable.

Chapter 8, on factory wastes and sewage follows the same general treatment as the preceding chapter. Preventive treatments consisting in the purification and precipitation of the harmful ingredients of factory affluents are suggested.

Of greatest interest, at least to the reviewer, is the chapter on virus diseases. It comprises about 200 pages of text and 40 illustrations. But even at that the treatment of many questions is all too short and the illustrations often insufficient. One notices especially the lack of good and clear drawings in the section dealing with cytological and anatomical disturbances incident to virus infection.

The chapter on virus diseases is divided into two parts. The first part deals with the general concept of virus diseases, their aetiology and symptomology. This account is followed by a study of disease transmission, the progress of the disease and its dependence on external factors; resistance, behavior of virus *in vitro,* classification of viruses and disease control. The second part deals with specific virus diseases of solanaceous and other plants. The section on the behavior of virus in the plant body deals with the nature of infection, the movement of virus in the plant body, the rapidity of this movement, and the concept of disease recovery.

Although not as amply illustrated, the second part of Volume I is withal a worthy companion to the first part. Its wealth of information and clarity of treatment make it profitable reading suitable, however, only in small doses. The language is simple and there is a gratifying lack of involved sentences; the technical terms are not always strictly German but of Latin or Greek derivation. The text is printed on exceptionally good paper and the binding assures greatest durability.—ERNST ARTSCHWAGER, U. S. Department of Agriculture, Bureau of Plant Industry, Sugar Plants, Washington, D. C.

REPORT OF THE TWENTY-SIXTH ANNUAL MEETING OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

THE PITTSBURGH MEETING

The twenty-sixth annual meeting of The American Phytopathological Society was held in Pittsburgh from December 27 to 29, 1934. The Cathedral of Learning, University of Pittsburgh, provided session rooms. Approximately 175 members were present and 110 papers were presented. In addition to regular meetings of the Society, joint sessions were held with Section G, and with the Mycological Society of America. The annual conference on Extension Work brought out much worth-while discussion with regard to the importance of close coordination between research and extension programs.

Abstracts of the papers on the program of this meeting were printed in the January number of PHYTOPATHOLOGY.

OFFICERS AND REPRESENTATIVES

The following officers and representatives were chosen:

President, H. T. Güssow, Central Experimental Farm, Ottawa, Ontario, Canada.

Vice-President, F. C. Meier, U. S. Department of Agriculture, Washington, D. C.

Secretary (three years), H. P. Barss, U. S. Department of Agriculture, Washington, D. C.

Treasurer and Business Manager (three years), H. A. Edson, U. S. Department of Agriculture, Washington, D. C.

Editor in Chief (three years), H. B. Humphrey, U. S. Department of Agriculture, Washington, D. C.

Councilor (two years), J. C. Walker, University of Wisconsin, Madison, Wisconsin.

Advertising Manager (one year), R. S. Kirby, Pennsylvania State College, State College, Pa.

Representatives on the Council of the A. A. A. S. (one year), N. E. Stevens, U. S. Department of Agriculture, Washington, D. C., and H. S. Cunningham, Long Island Vegetable Research Farm, Riverhead, N. Y.

Member of the Board of Governors Crop Protection Institute (three years), W. H. Martin, New Jersey Agricultural Experiment Station, New Brunswick, N. J.

Representative on Editorial Board, American Journal of Botany, G. W. Keitt, University of Wisconsin, Madison, Wisconsin.

The following temporary committees were appointed to serve throughout the meeting:

Auditing Committee, M. C. Goldsworthy and R. D. Rands.

Committee on Elections, C. E. F. Guterman and H. N. Brooks.

Committee on Resolutions, Annie R. Gravatt, Max W. Gardner, and G. R. Bisby.

REPORT OF THE SECRETARY-TREASURER, 1934

During the year 1933 the net loss in membership in the Society was 53, but for the year 1934 I am glad to be able to report an increase. We have had fewer resignations, fewer suspensions for non-payment, and more reinstatements of former members and applications from new members. We started the year with 774 members. We lost only 36 this year as compared with 84 in 1933. Two of our members died, 10 resigned and 24 were suspended for non-payment of dues. Seventeen former members were reinstated. The election of 43 new members at this meeting brings the total mem-

bership to 798, a net gain of 24. Of the total number, 102 are paid up life members and 68 are life-sustaining.

In this, my sixth and last report as Secretary-Treasurer and Business Manager, I wish to acknowledge the able assistance rendered during the first two years by Miss Mary G. Van Meter, of the Plant Disease Survey, U. S. Department of Agriculture, and during the last four years by my wife. With the increased time required for official duties, the active interest of Miss Van Meter and Mrs. Meier in the welfare of the Society, and their assistance with the work, was indispensable.

STATEMENT OF ACCOUNTS FOR THE YEAR ENDING NOVEMBER 30, 1934

Receipts:		
Balance from 1933		$1,782.00
Annual dues:		
1931	$5.00	
1932	35.00 ($20.00 life)	
1933	121.53 (39.83 life)	
1934	2,299.29 (339.35 life)	
1935	1,208.93 (270.04 life)	
1936	26.50 (10.00 life)	$3,696.25
Donations to Lyman Fund included in checks for dues		4.00
Sales included in checks for dues		1.60
Checks to replace those returned by bank		10.00
Total receipts		3,711.85
		$5,493.85
Expenditures:		
Member subscriptions transferred to PHYTOPATHOLOGY (1934, part 1931, 1932, 1933)		$2,870.42
Transferred to Sinking Fund Savings Account		400.00
Secretarial work		212.87
Printing (programs, ballots, bills, etc.)		75.80
Postage and stamped envelopes		41.36
Telephone and telegraph		8.81
Checks returned by bank and charge for collection of check		10.25
Expenses of Secretary-Treasurer, Boston meeting		46.35
Expenses of President, Boston meeting		66.20
Expenses of representative, National Research Council meeting		46.90
Expenses of Secretary-Treasurer, advance trip to Pittsburgh		3.65
Contribution for Biologists' Smoker, Boston meeting		15.00
A. A. A. S. registration fee for Journal Exhibit		1.00
Donations transferred to Lyman Fund		4.00
Sales transferred to PHYTOPATHOLOGY		1.60
Supplies		1.90
Federal tax on checks		0.58
Total expenditures		3,806.69
Balance on hand		1,687.16
		$5,493.85

SINKING FUND

The Sinking Fund, obtained by deducting $5.00 (formerly $6.00) from each life-sustaining membership payment, now stands at $8,321.00, of which $5,000 is invested in first mortgage notes. $3,181.00 is deposited in a savings account with the Riggs National Bank of Washington, D. C. The remainder, $140.00, has not yet been transferred from the checking account of the Society. The income from this fund now amounts to about $385.00 and is used to help defray the cost of publishing PHYTOPATHOLOGY.

Respectfully submitted,
F. C. MEIER, *Secretary-Treasurer.*

REPORT OF THE BUSINESS MANAGER OF PHYTOPATHOLOGY FOR 1934

STATEMENT OF ACCOUNTS FOR THE YEAR ENDING DECEMBER 4, 1934

Receipts:			
Balance from 1933			$1,067.64
Subscriptions: 1933	$ 63.55		
1934	2,838.36		
1935	252.17		
1936	1.00	$3,155.08	
Member subscriptions 1933 and earlier		237.30	
Member subscriptions 1934		2,633.12	
Sales of back numbers		312.98	
Special sale of 1933		84.00	
Advertising: 1933	$ 93.58		
1934	722.44	816.02	
Interest on 1st mortgage notes		299.50	
Interest on Sinking Fund Savings Account		119.69	
Grant from National Academy of Science		750.00	
From University of California for Esau illustrations		70.76	
Total receipts			8,478.45
			$9,546.09
Expenditures:			
Printing and Distributing PHYTOPATHOLOGY:			
Vol. XXIII, No. 11	$ 502.48		
Vol. XXIII, No. 12	415.79		
Index to Vol. XXIII	180.17	$1,098.44	
Vol. XXIV, No. 1	504.01		
No. 2	503.48		
No. 3	781.19		
No. 4	697.29		
No. 5	629.74		
No. 6	689.62		
No. 7	745.50		
No. 8	493.86		
No. 9	400.96		
No. 10	374.09	5,819.74	
Postage		607.77	$7,525.95

Secretarial work for Business Manager	221.87	
Secretarial work for Editor in Chief	258.50	
Miscellaneous expenses of office of Editor in Chief	43.20	
Secretarial work for Advertising Manager	41.34	
Commission and expenses of Advertising Manager	74.02	
Stamps and envelopes	49.24	
Telephone and telegraph	3.24	
Subscription refunded	6.00	
Preprints of abstracts for Boston meeting	21.59	
Insurance on back volumes stored at Univ. of Maryland	46.71	
Supplies and shipping material to Boston	1.40	
Printing	5.25	
Federal tax on checks	0.96	
Total expenditures		$8,299.27
Balance on hand		1,246.82
		$9,546.09

At the end of 1933 there were 502 non-member subscribers to PHYTOPATHOLOGY including 7 complimentary subscriptions. During the year there were 28 cancellations, and 11 suspensions due to non-payment. With the addition of 78 new subscribers, the net gain was 39, increasing the list to 541.

Respectfully submitted,
F. C. MEIER, *Business Manager.*

REPORT OF THE EDITOR IN CHIEF

With the closing of the current year, it is well to take stock of our Society's accomplishment as registered within the pages of our Journal PHYTOPATHOLOGY. As an indicator of research trends and progress in plant pathology, it is fairly reliable and, on the whole, a periodical in which the Society is fully justified in entertaining genuine pride, thanks to the continued improvement in the quality of the contributions submitted. On the other hand, we should be constantly aware of the fact that the need for continued improvement prevails.

Reports coming to us from home and abroad remind us that PHYTOPATHOLOGY is one of the best and most valuable journals of its kind in the world. Occasionally and too often, however, we receive complaints that, in brief, read or sound like the following: Too many of the papers are longer than is warranted by their essential substance; some of the contributions are of doubtful timeliness; not enough thought is given to selection of illustrative material; tabular matter, in many instances, should be better organized and condensed; manuscripts should be critically read by more people qualified to pass upon their merits; and so on, indicating that in the judgment of some of our readers, there still is room for further improvement.

The 24th volume of PHYTOPATHOLOGY contains 1386 pages of printed text and illustrations, or 380 pages more than appeared in Volume 23. The 1,386 pages of our journal for 1934 are classified as follows: Ninety-one articles, 58 phytopathological notes, 4 reports of regional or other meetings, 9 book reviews, 122 abstracts (4 by title only), and 263 text figures, and 3 plates. During the period January 1 to December 31, inclusive, approximately 153 manuscripts of articles, phytopathological notes, reports, book reviews, etc., and 175 abstracts (4 by title only) were submitted. Of this number 10 major manuscripts were returned to their authors for revision, 1 was found unaccept-

able, and 3 were withdrawn by authors. Of the manuscripts submitted in 1933, but too late for publication that year, 1 was withdrawn by the author and 2 were found unacceptable. In addition to the several papers published in 1934, 28 articles, 15 phytopathological notes, one book review, and 110 abstracts (1 by title only) are now (December 31) in press. The index for Volume 23 was published as a supplement to the April, 1934, number of PHYTOPATHOLOGY.

Summarized Report for the Triennium 1932 *to* 1934. The 22nd, 23rd, and 24th volumes of PHYTOPATHOLOGY comprise 244 articles, 124 phytopathological notes, 9 reports, 21 book reviews, 407 abstracts, 635 text figures, and 4 plates. The foregoing items comprised a total of 3394 printed pages, which, at an estimated average of $6.00 per page, cost the Society and a limited number of paying contributors, or supporting institutions, $20,364 for the triennium.

Of the total number of papers received, 31, or approximately 8 per cent, were returned to authors for revision; 16, or approximately 4 per cent, were found unacceptable and 8, or approximately 2 per cent, were voluntarily withdrawn.

The author wishes here to make grateful acknowledgment of the helpful service unselfishly rendered by the several members of the Editorial board, by Miss Frances W. Todd, and by the Science Press Printing Company.

Respectfully submitted,

H. B. HUMPHREY, *Editor in Chief.*

REPORT FOR PHYTOPATHOLOGICAL CLASSICS

FROM DECEMBER 8, 1933, TO DECEMBER 15, 1934

Total number of Classic No. 1 on hand		451
Number of Classic No. 1, sold,	58	
Number of gratis for advertising purposes	3	
Unaccounted for	1	
Total number disposed of		62
Balance on hand		389
Total number of Classic No. 2 on hand		656
Number of Classic No. 2 sold	63	
Number gratis for advertising purposes	3	
Unaccounted for	2	
Total number disposed of		68
Balance on hand		588
Total number of Classic No. 3 on hand		1020
Number of Classic No. 3 sold	216	
Number gratis for advertising purposes	3	
Number complimentary copies	9	
Unaccounted for	3	
Total number disposed of		228
Balance on hand		792

Classics sold as follows:

Classic No. 2	1 copy	@ $0.50		$0.50
Classic No. 3	155 copies	@ 0.50		77.50

Classic No. 1 & 2	2 copies	@	0.75	1.50
Classic No. 2 & 3	5 copies	@	1.00	5.00
Classic No. 1 & 3	1 copy	@	0.75	0.75
Classic No. 1, 2, 3	52 copies	@	1.25	65.00
Classic No. 1, 2, 3	2 copies	@	1.50	3.00
Classic No. 1, 2, 3	1 copy	@	2.00	2.00

Total	$155.25
Due on account	4.75
	$150.50
Plus Foreign Exchange	0.23
Total receipts for the current year	$150.73
Balance from last year	188.25
Total	$338.98
Expenditures:	
Printing of Classic No. 3	$121.40
Postage	10.07
Total expenditures	$131.47
Balance on hand December 15, 1934	$207.51

Respectfully submitted,
H. H. WHETZEL, *Business Manager.*

REPORT OF THE ADVERTISING MANAGER

During 1934 a total of 155 advertisements were run in PHYTOPATHOLOGY. This advertising consisted of a total of 108 pages. Of these, 50 were non-revenue, among which were exchange ads with other journals, meeting announcements and advertisements for Phytopathological Classics. The gross amount contracted for rose from $561.50 in 1933 to $1,000.45 in 1934, an increase of about 80 per cent. This upward trend is encouraging.

Respectfully submitted,
R. S. KIRBY, *Advertising Manager.*

REPORT OF COMMITTEE ON FOREIGN PLANT DISEASES

Your Committee submits for consideration the following report:

The Committee believes that The American Phytopathological Society, composed largely of men in public service and constituting a representative organization capable of forming intelligent opinion on the biological aspects of plant quarantine and restrictive measures, owes as a distinct duty to the nation to aid in the solution of the problems of guarding this country from foreign plant diseases. We recommend that the Council consider the advisability of scheduling a special session on quarantine problems at the next Annual Meeting. Among the numerous phases of the subject, the following appear to the Committee to be urgent and should be discussed at this or subsequent meetings:

1. The advisability of establishing a more adequate detention service through which questionable introductions may be safely held under observation.
2. The desirability of arranging for the testing of native plants under foreign

conditions to determine their reaction to foreign diseases not yet introduced here.

3. Your committee recalls a recommendation of December, 1927, on the desirability of establishing specialists in foreign countries to obtain specific information on foreign diseases, and strongly urges that, wherever possible, this policy be adopted by the administration.
4. The attitude that should be taken in regard to the exclusion of biological races of parasites already present in this country that might complicate the efforts here in breeding resistant plants or introduce more virulent parasitic types.
5. The safety measures that are needed in connection with the introduction of living cultures or viable specimens of parasites from abroad.

The American Phytopathological Society urges that in view of the serious situation presented by the Dutch elm disease special efforts should be made by state and federal authorities to extend search for the disease to all territory where the elms are of importance.

The Society further recommends that careful consideration should be given by the federal government and the states concerned to the feasibility of carrying out an eradication program against potato wart, as an alternative to continuing present quarantine measures or allowing the disease to spread.

Respectfully submitted,

C. R. ORTON, *Chairman,*

(See page 538 for Society action on this report.)

COMMITTEE ON NECROLOGY

During the calendar year 1934, there have been two deaths as follows:
Dr. Frank Lincoln Stevens, August 16, 1934.
Mr. Herman J. Ninman, October 24, 1934.

A. G. JOHNSON, *Chairman,*
G. P. CLINTON,
M. B. WAITE.

REPORTS OF OTHER COMMITTEES

Committee on Elections. The committee on elections, after having opened sealed-ballot envelopes and counting votes for each nominee, reports election of the following officers: President, H. T. Güssow; Vice-President, F. C. Meier; Secretary, H. P. Barss; Councilor, J. C. Walker.

C. E. F. GUTERMAN, *Chairman,*
H. N. BROOKS.

Auditing Committee. We have examined the books of the Secretary-Treasurer of the Society. In view of the fact that the above office is to be vacated, by the incumbent, at the end of the present year, a minute examination was conducted. We beg to advise the Society that we have found the accounts of the Secretary-Treasurer correct in all their details and that the books are in a most excellent order.

M. C. GOLDSWORTHY,
R. D. RANDS.

REPORT OF REPRESENTATIVES ON BOARD OF GOVERNORS, CROP PROTECTION INSTITUTE

During the year 1934 there were conducted 19 projects on part- or full-time basis. Of this total 7 were concerned directly with problems in plant pathology. All projects

were placed according to facilities and areas most adaptable for conducting the research and, where possible, conveniently accessible for the supporting company. The projects had headquarters or work conducted in the following states: New Hampshire, Illinois, Delaware, New Jersey, Massachusetts, Michigan, Iowa, Ohio, California, Connecticut, Florida, Virginia, Pennsylvania, Indiana, Missouri, and Washington. There were 5 publications issued in the Crop Protection Institute bulletin series. During the year 1934 the following representatives served as the Board of Governors: W. C. O'Kane, Chairman, Durham, New Hampshire; W. P. Flint, Illinois; Charles H. Richardson, Ames, Iowa; J. F. Adams, Vice-Chairman, Newark, Delaware; W. H. Martin, New Brunswick, New Jersey; I. E. Melhus, Ames, Iowa; H. J. Patterson, College Park, Maryland; W. H. MacIntire, Knoxville, Tennessee; E. D. Ball, Tuscon, Arizona.

The following summary presents the research and development projects directed by the Crop Protection Institute for the calendar year 1934:

Copper in Plant Nutrition. A study of increased yields brought about through the addition of copper salts to soils, including various typical soils not deficient in copper, and including a considerable number of important staple crops. Increased yields of economic importance have been secured. This project is financed by the Nichols Copper Company in cooperation with the Copper and Brass Research Association. Headquarters at the Delaware Experiment Station, but additional work carried on in various other States. Investigators employed include Raymond Russell, J. D. Hartman, W. L. Churchman and Woodrow Singles.

New Copper Fungicides. A project intended to develop new and better copper compounds that may supplement or take the place of those now in use. At least one new compound of unusual properties has been developed. This work financed by the Nichols Copper Company. Headquarters at the Delaware Experiment Station. Alexander Nikitin employed as investigator.

Oil Sprays. A continuation of a project in which improvements in the composition of oil sprays for summer application have been the objective. Promising results have been secured. This project financed by the Standard Oil Co. of Indiana. Headquarters at the Illinois Experiment Station. Investigator employed on the project, John L. Alsterlund.

Pyrethrum Culture in the United States. A continuation of a thorough and extensive study intended to solve the various problems involved in commercial growing of pyrethrum in the continental United States. Good progress has been made and a solution has been arrived at for various problems involved in the domestic production of this important plant. This project supported by Stanco Incorporated. Headquarters at the New Jersey Experiment Station, with extensive supplemental work in Pennsylvania. Investigator employed, R. E. Culbertson.

Preliminary Study of Organic Compounds of Promise as Insecticides and Fungicides. An exploration of various organic compounds which may be of value in the development of new and better sprays. Supported by the Monsanto Chemical Company on a part-time basis. Headquarters at the New Hampshire Experiment Station. Investigator employed, W. A. Westgate.

Improvement in Pyrethrum Sprays. Continuation of a project designed to develop more efficient ways of using the active principles of pyrethrum flowers. A new and improved method of using the principles has been discovered. This work financed by J. C. Makepeace. Headquarters at Wareham, Mass., with supplemental work in Florida and New Hampshire. Investigator employed, George L. Walker.

Development of a New Spray for Codling Moth and Other Insects. An investigation of a considerable series of new organic compounds among which several have been discovered which are giving promising results and one in particular which has reached

the point of successful field use. This work financed by the Dow Chemical Company. Headquarters at the Iowa Experiment Station, with supplemental work in Michigan and further work in various other States. Investigator employed, John F. Kagy.

New Fumigants. Continuation of a project utilizing carbon dioxide in conjunction with other materials for the fumigation of stored products. A new principle in utilization of carbon dioxide has been discovered, and a new mixture of gases developed which is now on the market under the name Proxate. The fumigant is safe for the operator, is not explosive, is inexpensive, and is giving consistently satisfactory results. Headquarters at the Iowa Experiment Station and in Chicago. Investigator employed, R. M. Jones.

New Fungicides and Insecticides. A study of a long series of organic compounds that has resulted in the development of at least one material of promise. This project suspended at present awaiting manufacturing facilities. Supported by the National Aniline Company. Headquarters at the Delaware Experiment Station. Investigator employed, C. N. Priode.

New Contact Insecticide. An investigation intended to develop a means of utilizing Halowax oil as the active component of a new contact insecticide and resulting in the invention of a new material which is giving excellent results and has advanced to semi-commercial use. Supported by the Halowax Corporation. Headquarters at Ohio State University. Investigator employed, E. P. Breakey.

Sulphuric Acid in Weed Control. A study planned to disclose satisfactory methods of utilizing dilute sulphuric acid in control of weeds in cereals, notably mustard in barley, together with means of utilizing concentrated sulphuric acid in combination with other substances for eradication of resistant weeds. Machinery for application of dilute sulphuric acid has been devised and results of economic importance have been secured. This investigation financed by the Freeport Sulphur Company. Headquarters at the Davis Substation of the University of California. Investigator employed, Willis E. Ball.

Development of New Insecticides and Fungicides Utilizing Sulphur and Carbon Disulphide. A thorough study of the possibilities of these two substances, both alone and in combination, and both from the insecticidal and from the fungicidal point of view. Financed by the Stauffer Chemical Company. Headquarters at the Connecticut Experiment Station and the Massachusetts Experiment Station. Investigators employed, M. V. Anthony, and T. T. Ayers.

Plant Extracts, Including Pyrethrum, Derris, and Others. A project planned to develop better means of utilizing the active principles of various plant extracts in insect control. Supported by S. B. Penick & Company. Headquarters at the New Hampshire Experiment Station and in Southern States. Investigators employed, O. J. Smith and W. C. Baker.

Cattle Sprays. A study of the composition of cattle sprays and their improvement. Financed by the Hercules Powder Company. Headquarters at the Delaware Experiment Station. Investigator employed, A. M. Pearson.

New Spray Materials and New Means for Their Application. A project planned to develop improved methods of application of spray materials in greenhouses, home gardens, and similar situations, together with suitable materials for the new device. Supported by the Mergenthaler Linotype Company. Headquarters at the new New Hampshire Experiment Station. Investigator employed, William H. O'Kane.

Codling Moth Sprays. A project for the development of new and safe spray materials for codling moth control. One or more materials of promise have been discovered. Headquarters at the Delaware and Indiana Experiment Stations, with sup-

plemental work in Virginia, Missouri, and Washington. Supported by the General Chemical Company. Investigator employed, Merrill M. Darley.

Respectfully submitted,

J. F. ADAMS,
I. E. MELHUS,
W. H. MARTIN.

Report of the Committee on Resolutions. The American Phytopathological Society wishes to thank the American Association for the Advancement of Science for making general arrangements for these meetings. It is also grateful to Prof. O. E. Jennings of the University of Pittsburgh and his associates for arranging many details.

To the management of the Hotel William Penn The American Phytopathological Society wishes to express its appreciation of the many courtesies extended during these meetings. Special thanks are due Mr. Gerald P. O'Neill, General Manager, and Mr. Sam K. Smith, Sales Manager.

Applause and appreciation are due R. S. Kirby, A. L. Pierstorff, F. J. Schneiderhan, and W. H. Thurston, the committee that arranged the entertainment at the annual dinner. Delightful interludes were afforded by the accordian and vocal solos of Miss Leon Metzger.

Sincere appreciation is extended to F. C. Meier, Secretary-Treasurer of the Society and Business Manager of PHYTOPATHOLOGY for six years of efficient and unceasing service, and to H. B. Humphrey for his continued efficient editorship of PHYTOPATHOLOGY.

ANNIE R. GRAVATT,
MAX W. GARDNER,
G. R. BISBY.

ACTION OF THE COUNCIL

In addition to making the appointments of the officers mentioned earlier in this report, the Council submitted the following actions, which were approved by the Society:

1. It is recommended that the Editor in Chief of PHYTOPATHOLOGY be authorized to recommend to the Council the necessary associate editors for 1935.
2. It is recommended that the Editor in Chief of PHYTOPATHOLOGY be allowed actual expenses up to or within $350.00 for secretarial and editorial assistance during 1935.
3. It is recommended that the incoming Secretary prepare a revised membership list and revised constitution, and that the Treasurer and Business Manager be authorized to pay for printing these.
4. It is recommended that the Secretary-Treasurer be authorized to pay part expense ($49.50) incurred by our representative in travelling to the 1934 meeting of the National Research Council.

CONSTITUTION AMENDED

In accordance with provisions of Article X, the Society voted to amend the Constitution by discontinuing the office of Secretary-Treasurer, and establishing the separate offices of Secretary and Treasurer, each with a three-year term. Under Standing Rules it was voted to increase the term of office for the Business Manager of PHYTOPATHOLOGY from 1 to 3 years.

OTHER BUSINESS

The reports of officers, committees, and representatives as printed above were accepted. Action of the Council as indicated above was approved by the Society.

At the request of the Council, Dr. Donald Reddick reported in detail on the steps taken and progress made during the past five years toward the formation of some type of international organization of plant pathologists. In line with previous resolutions of the Society, the Council was authorized to effect a union with the Phytopathological Section of the International Union of Biological Societies, at such time and in such manner as may be practicable.

Reading of the report of the Committee on Foreign Plant Diseases was followed by a motion, made by Dr. E. C. Stakman, to amend the report as it relates to the Dutch elm disease as follows:

The Society also wishes to commend the Department of Agriculture and the cooperating States for the efforts now being made to investigate the disease and control or eradicate it. The Society wishes to urge, furthermore, that the Department do everything in its power to learn all of the pertinent facts in connection with the disease and to make every reasonable attempt to control or eradicate it.

After discussion of the amendment and the need for an active program for control of the Dutch elm disease, the amendment was passed and the chairman of the committee was instructed to present in person the Society's action relating to the Dutch elm disease and the potato-wart disease to the Secretary of Agriculture, the Chief of the Bureau of Plant Industry, and the Chief of the Bureau of Entomology and Plant Quarantine. (This was done by C. R. Orton on January 29, 1935).

A motion made by Charles Chupp that the Council reorganize and expand the membership of the Committee on Extension to include research representation was carried. The Society also passed a motion to devote a session at the next annual meeting to a discussion of the coordination of research and extension.

PHYTOPATHOLOGY

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PATHOLOGICO-ANATOMICAL CHANGES IN THE TOMATO INCIDENT TO DEVELOPMENT OF WOODINESS OF THE FRUIT[1]

P. V. MICHAILOWA

(Accepted for publication May 23, 1934)

Woodiness of tomato fruit has been known in the Crimea and Australia for many years. In August, 1933, it was simultaneously described by Samuel, Bald, and Eardley (6), on the one hand, and by Rischkow, Karatschewsky and Michailowa (4) on the other. This disease, which was described (4) as "fruit woodiness," is known in the Crimea under the name of *stowboor,* while in Australia it is called big bud.

In our preliminary report (4) we described the characteristic anatomical features of plants affected with this disease; some of them also are mentioned in the description given by the Australian authors. In this paper we present a more detailed analyses of histological changes observed in *stowboor,* which form the most interesting features of this disease.

ANATOMY OF THE TOMATO PLANT

Before describing the anomalies in the structure of the stalk, let us consider briefly the structure of a normal tomato stalk. If we examine a cross section of a young tomato stalk from the periphery to the center, we shall note the following: an epidermis of a single layer of cells and assimilating tissue, 3 or 4 cells deep, rich in chloroplasts that lend their green color to the stalk. Next to the assimilating tissue lies the collenchyma, comprising 6 or 7 layers of thick-angle cells. Then we note the cortical parenchyma, a single-cell thickness of endodermis with a great many starch grains. Beyond these structures lie the bicollateral fibrovascular bundles.

The xylem consists of vessels, tracheids, and a little libriform. During the growth of the tomato the wood tissue gains more and more in mass, forming a closed ring. In the phloem there are sieve tubes, satellite cells, phloem parenchyma, and, in older stalks, also, thick bast.

The center of the stalk consists of the big pith-cells. The parenchymatous cells of the pith adjoining the inner phloem contain a few grains of starch.

[1] This investigation was conducted in the Laboratory for Virus Diseases, Ukrainian Institute of Plant Protection (U. S. S. R., Kharkow, Sozselchos House).

Having considered the structure of a normal stalk we may analyze the changes occurring as a result of the woodiness of fruits. If the plant be attacked by the disease at an older age, the part of the stalk that had developed before the plant became infected in no way differs from a healthy plant. Below are recorded the results of an investigation of both a normal and a diseased plant.

A part of the stalk measuring 28 cm. from the top was investigated. Twenty-eight cm. below these the cuts of the normal and the diseased stems were quite identical, for, during the development of these parts of the stalk, the tomato plant had evidently been healthy. The structure of the diseased stalk, as described below, is typical for all stalks characterized by woodiness of fruit.

Normal Plant	*Diseased Plant*
(1) A cross section made 0.5 cm. below the top. The fibrovascular bundles are hardly visible. The xylem consists of a small number of vessels. They lie in the bundle in 1 or rarely 2 rows along the radius. The inner and exterior phloem occupies but little space in the bundles. Starch is found only in the cells of the bundle sheath (endodermis).	(1) In a cross section made 0.5 cm. from the top, the bundles appear to be perfectly developed. The vessels are arranged in 2–3 rows, seldom in 1 row, along the radius. Their number is nearly twice as great as in the normal plant. The inner phloem occupies enormous areas in the bundles. The cells of the sieve tubes are filled with a yellowish substance of unknown nature. Numerous starch grains are present in the cells of the pith, collenchyma, and cortical parenchyma.
(2) At 3 cm. below the top, the activity of the interfascicular cambium is evident, its cells are just beginning to divide, the space between the bundles is occupied by parenchymatous cells. The phloem occupies little space, small groups being visible in the neighborhood of the xylem. Starch only is contained in the endodermis. The fibrovascular bundle looks like that belonging to a diseased plant from 0.5 cm. from the top upwards.	(2) At 3 cm. from the top, the fibrovascular bundles are better developed than those of a healthy plant. The ring of wood tissue is plainly visible. The inner phloem is very broad, forming nearly continuous rings; much starch is present.
(3) At 5 cm. from the top, the same as in the foregoing section. Bundles are very poorly developed; inner phloem occupies only a small part of the bundles. Starch present in the bundle sheath.	(3) 5 cm. The fibrovascular bundles are thicker than in the norm. The ring of xylem is distinctly visible. A very broad inner phloem. Much starch.
(4) At 8 cm. interfascicular xylem with slightly thickened walls has appeared. The inner phloem occupies but little space in the bundles. A few starch grains are met with in the parenchyma, the pith, and also in the bundle sheath.	(4) 8 cm. Bast is present in separate areas in the exterior phloem. The mechanical elements of the interfascicular xylem have thicker walls than in the healthy plant. The inner phloem occupies much greater expanses than in the normal or healthy plant. Much starch in all tissues.

(5) At 10 cm. the differences between the healthy and the diseased stalk are, on the whole, the same as in the former sections. The structural make up recalls that shown in the cross section made at 8 cm.

(6) At 14 cm. from the top, bast cells are observed here and there. The number of vessels in a bundle is smaller than that in a diseased plant. The quantity and position of starch grains are the same as in the preceding sections.	(6) 14 cm. Bast in the shape of a solid ring, especially distinctly visible near the great fibrovascular bundles. The medullary cells are crowded with starch grains.

(7) At 18 cm. each fibrovascular bundle contains from 63 to 94 vessels. The bast forms a solid ring, although it is not seen in such strong relief. The interfascicular xylem is 0.11 to 0.099 mm. broad. The inner phloem occupies much smaller areas and is present in much smaller quantities than in a diseased stalk. Starch grains are as numerous as in the section made at 8 cm.

(8) At 23 cm. the structure reminds one of that of the foregoing section, only the bast cells are more numerous and more plainly visible.

(9) At 28 cm. the width of the vessels is the same in healthy as in diseased plants. The xylem measures 0.22 mm. in the broadest and 0.099 mm. in the narrowest places. The exterior phloem has one layer of thick bast, which forms a solid ring. In some places bast also is contained in the inner phloem.

(7) At 18 cm. there are 80 to 130 vessels per bundle. The solid bast ring plainly divides the cortical parenchyma from the exterior phloem. The interfascicular xylem is 0.099 mm. to 0.154 mm. broad. The inner phloem forms an almost solid ring inward from the xylem. Very numerous starch grains.

(8) At 23 cm. the structure is markedly like that of the section made at 18 cm. In the inner phloem some few small areas of bast have just appeared.

(9) At 28 cm. the cross sections of vessels look like those of healthy stalks. The interfascicular xylem is better developed than in the healthy plant. The maximum breadth of xylem is 0.264 mm., the minimum, 0.132 mm. In the structure and position of the bast cells the exterior phloem in no way differs from that of a healthy tomato plant. As to the inner phloem, its development is much stronger than the inner phloem of a normal plant or exterior phloem of a diseased plant. The parenchyma of the pith and cortex contains many starch grains.

In summing up the results of the study of the stalk, the following facts should be noted: (1) Under the influence of the virosis the plant develops much more rapidly than a corresponding healthy one. The development of the tissues of healthy tomato plants seems to lag in comparison with that of the diseased ones. For instance, the fibrovascular bundles of a tomato suffering from woodiness of fruit appear to be quite developed at a distance of about 0.5 cm. from the top of the stalk, if the plant be infected; whereas such development occurs only at a distance of 3 cm. from the top if the plant be healthy. The cambium of the diseased plant begins to form xylem and phloem at a distance of 3 cm. from the top, whereas in a healthy individual, this development would occur only at a distance of 8 cm. from the top; bast appears at a distance of 8 cm. in diseased and of 14 cm. in healthy plants.

Moreover, it is observed that xylem cells of a diseased individual earlier acquire a woody consistency than do those of a normal plant. (2) The interfascicular xylem begins to form earlier and develops at a greater rate when the plant is suffering from the disease. At a distance of 28 cm. from the top in a normal plant the xylem measures only about 0.165 mm. in diameter, while in a diseased plant this tissue attains a diameter of 0.198 mm. The number of vessels is twice as great in a diseased plant as in a normal one. (3) The structure of the exterior phloem shows no great difference except that the formation of bast begins earlier and the sieve tubes of a diseased stalk show peculiar contents. As long as the interfascicular xylem has developed no partitions between the bundles, the inner phloem

of a diseased tomato plant occupies more space than the rest of the bundle and is, of course, much more strongly developed than it would be in a healthy plant. The cells of the sieve tubes are filled with a yellowish substance of unknown nature. (4) Of great interest is the difference in the storing of starch grains. In a healthy stalk, starch is found in the starch-bearing layer (bundle sheath) and a little of it is contained in the pith cells surrounding the inner phloem. In the stalk of a tomato plant attacked by this disease, starch is found in the parenchymatous cells of the medulla, the cortex, and the collenchyma. Where starch is found, it is present in such great quantities that the cells are gorged with it. Elements of wood tissue are often observed in the region of the inner phloem and the medulla of diseased plants. The cells of mechanical tissue give a positive reaction to the lignin test and stain like lignin. These elements occupy different spaces. In the inner phloem there is not much of the same, but in the medulla the wood elements form $\frac{1}{4} - \frac{2}{3}$ of the medullar space (Fig. 1, B).

THE LEAF BLADE

A cross section of a healthy leaf shows the upper epidermis, consisting of cells of different sizes and shapes; palisade parenchyma formed of one layer, the ends of the cells of which adjoining the spongy parenchyma are sharp; spongy parenchyma with wide intercellular passages; and lower epidermis of the same structure as the upper one (Fig. 2, A).

The cross section of a leaf of a tomato plant infected by this disease shows a different structure. The upper epidermis is formed of a compact layer of cells of nearly uniform size. Beneath them is the palisade parenchyma with cells that are short in comparison with those of a healthy palisade parenchyma.

The spongy parenchyma consists of 3 layers. Its cells lie close to each other with hardly any intercellular spaces between them. The lower and upper epidermises are structurally the same (Fig. 2, B).

Carbon assimilation is much more intense in the leaves of a normal plant than in a diseased one. The test with chlorohydrate iodide, used to determine the presence of starch, shows that sections of a healthy leaf contain more and larger starch grains.

PETIOLE

Cross sections of the petioles of healthy and diseased tomato leaves show a difference in xylem structure. The entire section through a petiole of a healthy leaf has 75 to 79 vessels, while that of a diseased plant shows as many as 123 to 151. The width of the vessels varies. A vessel of a diseased plant measures 0.036 to 0.0135 mm.; that of a healthy plant 0.0505 to 0.0198 mm. The relative values of the inner and outer phloem are the

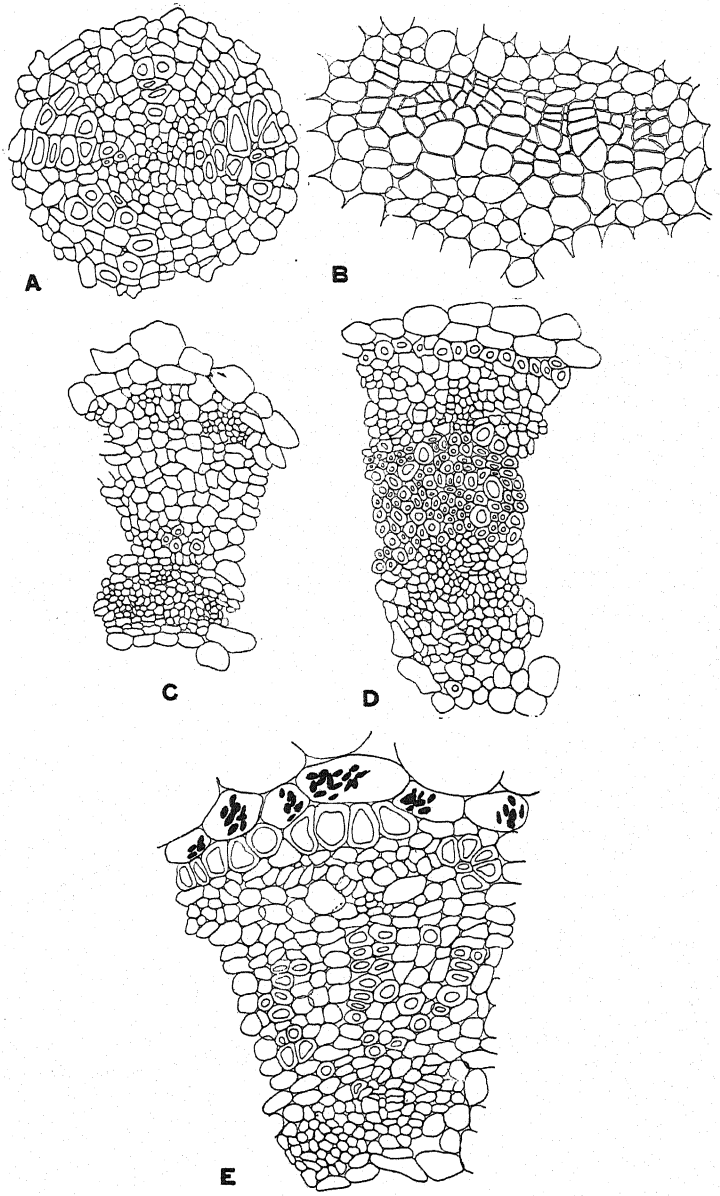


FIG. 1. A. Cross section of fibrovascular bundle of a normal tomato fruit. B. Cross section of stalk of a diseased plant, showing wood tissue of the pith. C and D. Fibrovascular bundles of fruit stalk when tomato fruit was just showing. C. Normal. D. *Stowboor*. E. Cross section through the leaf trace from sepal on the peduncle of a *Stowboor* tomato plant.

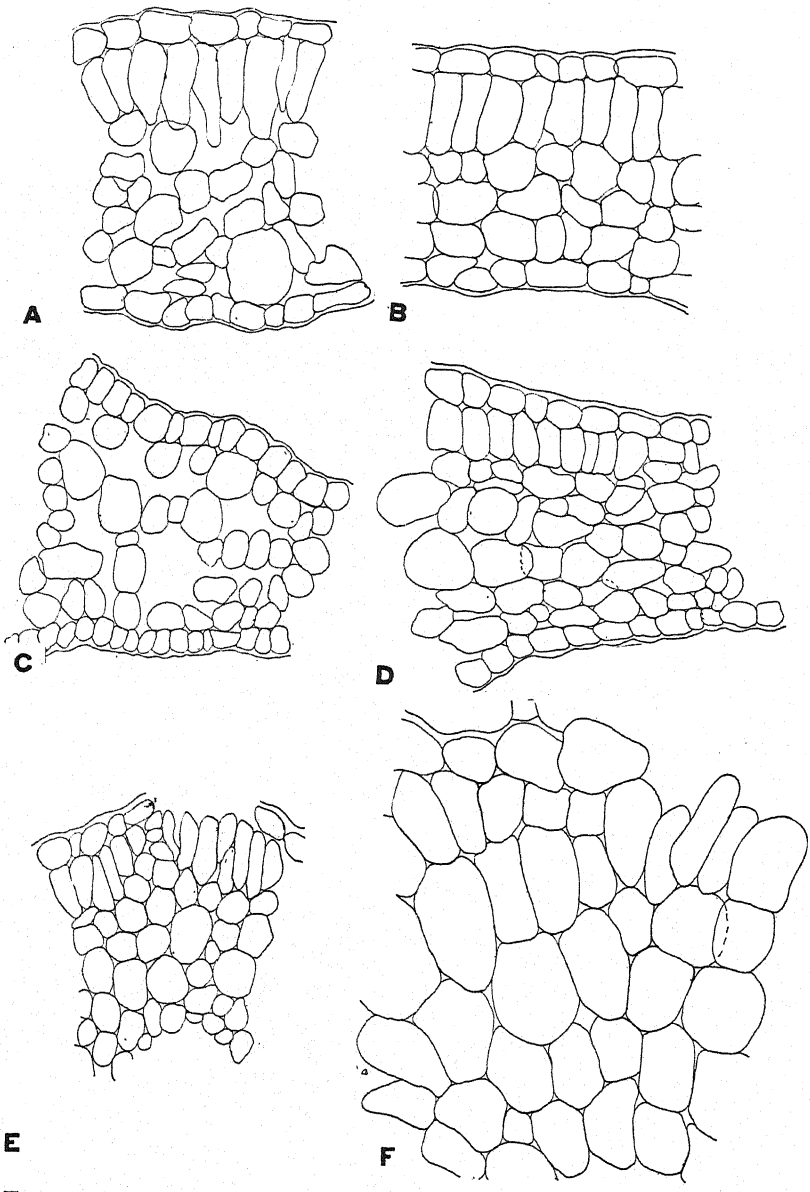


FIG. 2. A and B. Cross sections of tomato leaf. C and D. Cross sections of tomato petal. E and F. Cross sections of tomato sepal. A, C, and E. Healthy. B, D, F. *Stowboor.*

same as for those of the stalk. The inner phloem of the diseased plant is much better developed and the sieve tubes and companion cells are filled with a yellow substance.

PEDUNCLE AND PEDICEL

Schwarz (7) has studied the structure of both the peduncle and the pedicel of the tomato. He performed his anatomical investigation with a view to determining what connection, if any, existed between the development of the tissues of a fruit and that of the tissues of its pedicel.

Let us first consider the anatomical structure of the peduncle and then that of the pedicel. It must be noted that material serving for these inves-

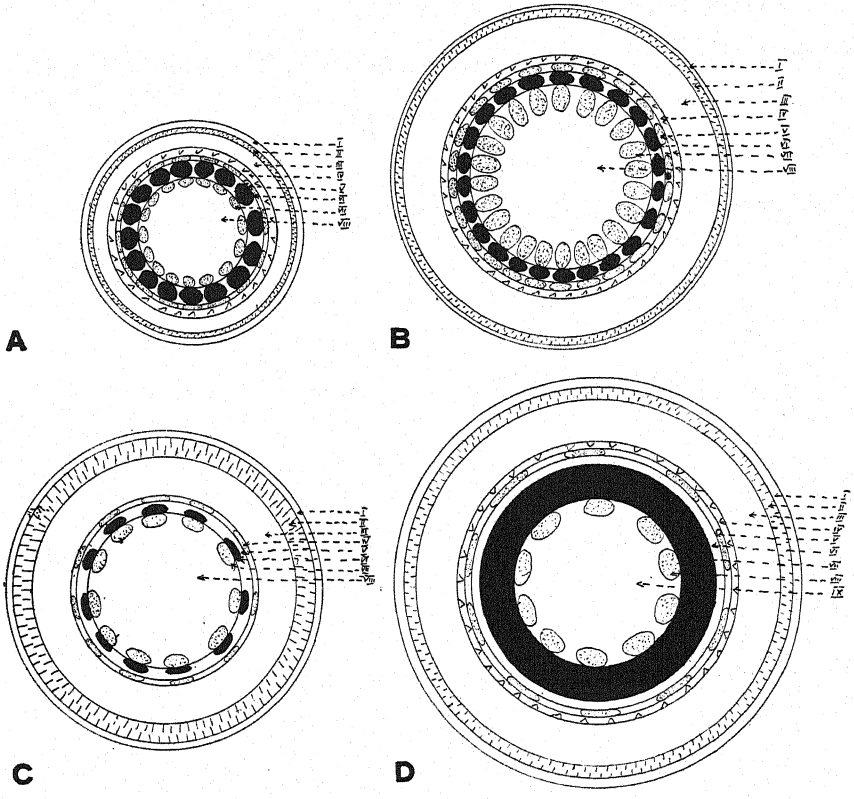


FIG. 3. A and B. Diagrams of tomato peduncle. C and D. Diagrams of tomato fruit stalk when fruit was just beginning to form. A and C. Normal. B and D. *Stowboor*. In both A and B: I, epidermis; II, assimilating tissue; III, collenchyma; IV, cortical parenchyma; V, outer phloem; VI, xylem; VII, inner phloem; VIII, pith. In both C and D: I, epidemis; II, assimilating tissue; III, cortical parenchyma. In C: IV, outer phloem; V, cambium; VI, xylem; VII, inner phloem; VIII, pith. In D: IV, bast; V, outer phloem; VI, cambium; VII, xylem; VIII, inner phloem; IX, pith.

tigations was always taken from fruits and flowers of the same age, and the sections were made at the same distance from the top in diseased as in healthy plants. The peduncles were those of flowers whose corollas were open.

The section through a healthy peduncle shows epidermis, 2 layers of assimilating tissue, 3 layers of collenchyma, and nearly as many of cortex parenchyma. The fibrovascular bundles were of bicollateral type, the inner and outer phloems were nearly equal to each other in volume and development. The xylem consisted mainly of vessels and libriform cells. There is considerably more of it than there is of the phloem. The center of the section is occupied by medullary parenchyma. The diameter of the peduncle is about 1.44 mm. (Fig. 3, A).

The cross section of a diseased peduncle shows the same tissues as those of a healthy plant, with this difference, however, that the development of these tissues is richer. The collenchyma consists of 4 to 5 layers, its cells being much bigger; therefore, if the collenchyma of a healthy plant does not exceed 0.066 mm. in breadth, it measures about 0.198 mm. in the peduncle of a diseased plant. The same might be said of the pith, its diameter being 0.462 in healthy and 0.671 in diseased peduncles. The inner phloem requires special attention. As is shown in figure 3, B the area occupied by the inner phloem nearly equals that of the xylem and the outer phloem combined, so that the inner phloem is nearly twice as wide as the outer. The areas of the inner phloem proliferate so much that they form an almost solid ring. The whole peduncle measures 1.793 mm. in breadth.

During the growth of the fruit the difference becomes greater and greater, as is shown by figures 4, A, B, and 3, C, D.

While the fibrovascular bundles are plainly visible in a healthy fruit stalk when the fruit is just starting on its growth (Fig. 3, C and 1, C), in a diseased fruit stalk there is a solid ring of xylem (3, D and 1, D). In the outer phloem of a diseased fruit stalk, bast has formed and surrounds the whole fruit stalk like a ring. The fruit stalk of a diseased plant is about 1 mm. thicker than that of a healthy one, while the fruit itself measures 4 cm. in diameter. The diameter of a healthy fruit stalk is about 2.739 mm., while in a diseased plant it is approximately 3.74 mm. The thickening is due to the development of all the tissues of the fruit stalk (Fig. 4, A and B) but especially to that of the medulla, which has bigger cells in a diseased plant, measuring 1.628 mm. instead of 1.265 mm. in the pedicel. The inner phloem of the pedicel of a diseased plant has a radius of 0.209 mm. instead of 0.099 mm., as in a normal plant. The xylem of the former attains a radius of 0.385 mm. instead of 0.253 mm., and the outer phloem, which measures 0.044 mm. in the pedicel of a healthy plant, attains a radius of 0.099 mm. in that of a diseased one.

In summing up we note that the phenomena observed in both the peduncle and pedicel are the same as those that obtain in the stalk. We see that the tissues of a healthy tomato plant are not growing so fast nor is the development of the tissues so vigorous as that of a diseased plant. The hypertrophy of the bundles is plainly visible in each section, a fact that deserves comparison with the observations made by Schwarz (7), who studied the anatomical structure and solidity of the peduncles of heavy

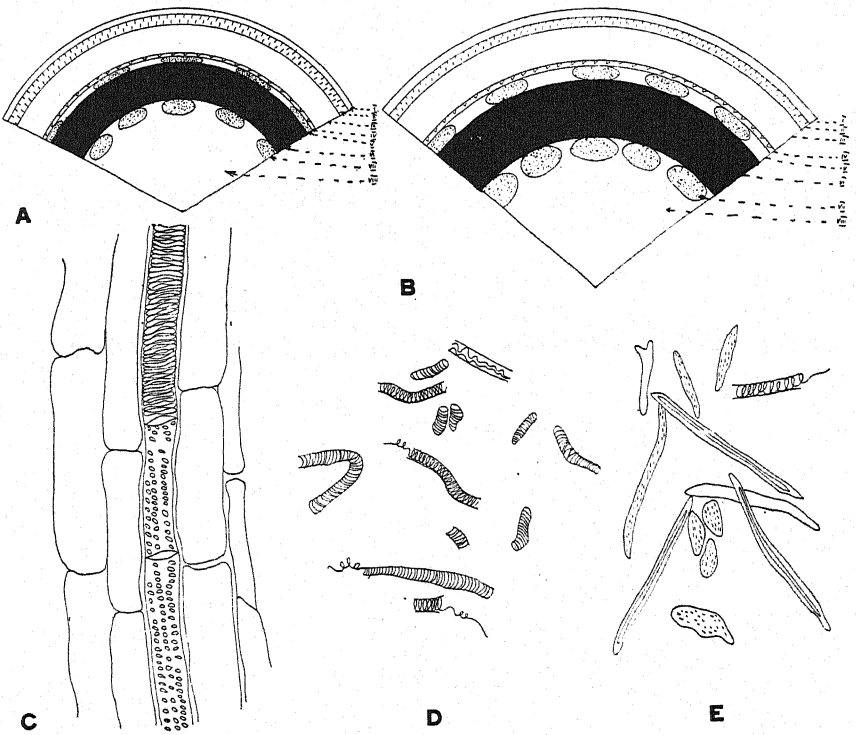


FIG. 4. A and B. Diagrams of old fruit stalks of tomato. A. Normal. B. *Stowboor*. In both A and B: I, epidermis; II, assimilating tissue; III, cortical parenchyma; IV, bast; V, outer phloem; VI, xylem; VII, inner phloem; VIII, pith. C. Spiral vessel changed to porous one. D and E. Macerated fibrovascular bundles of tomato fruit. D. Normal. E. *Stowboor*.

fruits, including the tomato. He proved that, contrary to the prevailing opinion of the great practical perfection of the development of mechanical tissue in the pedicel, this tissue shows a development several hundred times greater than that necessary to sustain these heavy fruits. Their firmness and strength are far in excess of the safety quotient required by the rules of engineering. Even the peduncles of the flowers are sufficiently strong, according to Schwarz (7), to sustain the future fruit, so that the develop-

ment of their mechanical tissues, which is correlated to the growth of the fruit, may hardly be considered necessary. We found that, owing to the virus, the pedicel becomes even stronger, although the fruits of plants suffering from this disease generally are smaller than normal ones. This fact proves that solid mechanical structures "perfectly answering the demands of engineering" may be the result of a pathological agent, and not a kind

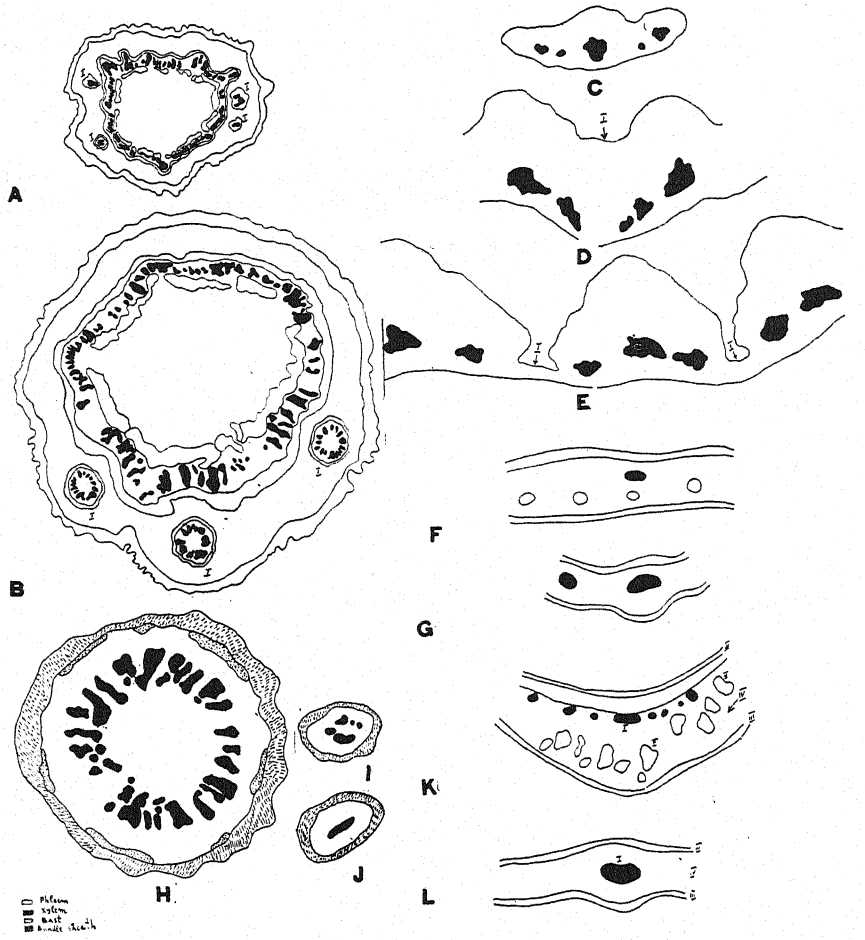


FIG. 5. A and B. Diagrams of tomato peduncle. A. Normal. B. *Stowboor*. In both A and B: I, leaf trace from sepal. C to E. Diagrams of cross sections through sepals. C. Normal. D. and E. *Stowboor*. F. and G. Diagrams of cross section of a Convolvulus petal. F. Normal. G. *Stowboor*. H to J. Leaf traces from sepals in the peduncles of tomato. H. *Stowboor*. I and J. Normal. Explanation in left corner of H applies also to I and J. K and L. Diagram of cross section of a Convolvulus sepal. K. Normal. L. *Stowboor*. In K and L: I, fibrovascular bundle; II, intercellular spaces; III, epidermis; IV, mesophyll.

of adaptable reaction. It is unwise to conclude that a seemingly adaptable structure has indeed an adaptable function.

The following fact deserves special attention. If we cut the peduncle of a diseased tomato plant 0.5 to 1 cm. below the flower we find a certain number of complementary steles near the epidermis, each stele being surrounded by a bundle sheath of its own. These steles are formed by the leaf trace of the sepals that stretch through the cortex of the peduncle. The number of such steles equals that of the fibrovascular bundles beginning in the sepals, and the cross section of the peduncle is structurally similar to that of the stalk of polystelic type (Fig. 5, B.) After the cross section had been treated with phlorglucin + concentrated HCl solution, the tissues of these complementary steles could be observed in greater detail. The center of the cross section (Fig. 5, H) was almost wholly filled with inner phloem.

The inner phloem was surrounded with xylem elements radially disposed. The xylem is followed by the exterior phloem, which, even in the peduncle, contains areas of bast. The layer quite at the periphery and separating the leaf-trace from the other tissues of the peduncle is an independent bundle sheath.

These findings forced us to consider carefully the structure of a normal peduncle in order to see whether there could be found there comparable structural phenomena. Indeed, the healthy peduncle was observed to contain traces of the same structure, but these were most insignificant. Only just beneath the sepal the cortex of the peduncle showed leaf-traces, each of the latter being surrounded by its own sheath. Very soon they joined the axial cylinder. These formations, occupying such a short stretch of space, were but slightly developed (Fig. 5, A and J).

The xylem occupied a small area, the inner phloem was hardly visible or even utterly lacking, and the outer phloem had no thick bast. It is known that polystely in dicotyledons occurs most rarely. It is interesting to note that, according to Solereder (8), polystely may be found in the pedicel of some Guttiferae, Terustroemiaceae, Malvaceae, Sterculiaeae, Meliaceae, and Moraceae.

THE FLOWER

We shall begin with the structure of the calyx. Figures 5, C, D, E and 2, E, F represent the corresponding areas of sepals of healthy and diseased flowers. Figure 2, E, F shows that the thickening of the sepals is not the result of an increased number of cells, but is caused by their greater volume. Passing now to the petals, we find the mesophyll is structurally very loose and undifferentiated in a healthy tomato plant. The petal of a flower attacked by woodiness almost completely lacks intercellular spaces or has very re-

stricted ones. The tissues are differentiated. The mesophyll cells are disposed lengthwise and remind one of the palisade parenchyma of the leaf. The rest of the tissue has cells of different shape that are closely packed together (Fig. 2, D). Such a development of the palisade tissue of the petal mesophyll may be regarded as the first stage of antholysis and transformation of the petals into leaves.[2]

The cross section through this axis of the flower above the sepal shows that the cells of the medulla, parenchyma, cortex, and collenchyma are crowded with grains of starch. The leaf traces of the petals have the same structure as the leaf traces of the sepals. For a certain time they form independent cylinders in the cortex. The difference is that of the duration and degree of development. The leaf traces of the petals are much shorter than those of the sepals. The leaf trace of a sepal is about 10.5 mm. long; that of the petal measures 4.5 mm. The diameter of the leaf traces of the sepals exceeds that of those of the petals.

THE POLLEN

The pollen of diseased plants deserves special attention. Having examined a great many pollen grains from the anthers of various flowers we did not find a single one of normal appearance. All of the pollen was sterile and without contents. The wall was shrivelled; often it was collapsed into a small lump. The normal pollen grain is of granular structure, spheroid, and possesses 3 spots through which pollen tubes grow. The structures of the anthers themselves show also appreciable differences. (Fig. 6, A and B).

THE FRUITS

The fruits suffer more than all the other parts of the plant. They are not suitable for food because of the strong lignification of the fibrovascular system; they become also quite tasteless.

Such fruits are easily distinguished from normal ones. They never turn quite red. Generally they are yellow with an orange or pinkish hue. In green fruit one may often find diaphanous spots formed by tissues filled with water, measuring 2×1.5 mm. or even more. The fruits are ribbed. In cross section a single fruit presents the following appearance: The placenta is quite free of pigmentation, being white. Only the peripheral parts of the pericarp are red, while the central parts remain as white as the placenta. The fibrovascular bundles are brown and so hardened that they can be plainly felt as one rubs the cut surface with the fingers. If one press the exposed cut ends, one experiences even a pricking sensation. A healthy tomato, when cut, is uniformly red; the fibrovascular bundles are white and just as soft as the cells of the placenta.

[2] It is known that woodiness of the tomato often lengthens the axis of the flower beyond the calyx. (Fig. 2, C and D.)

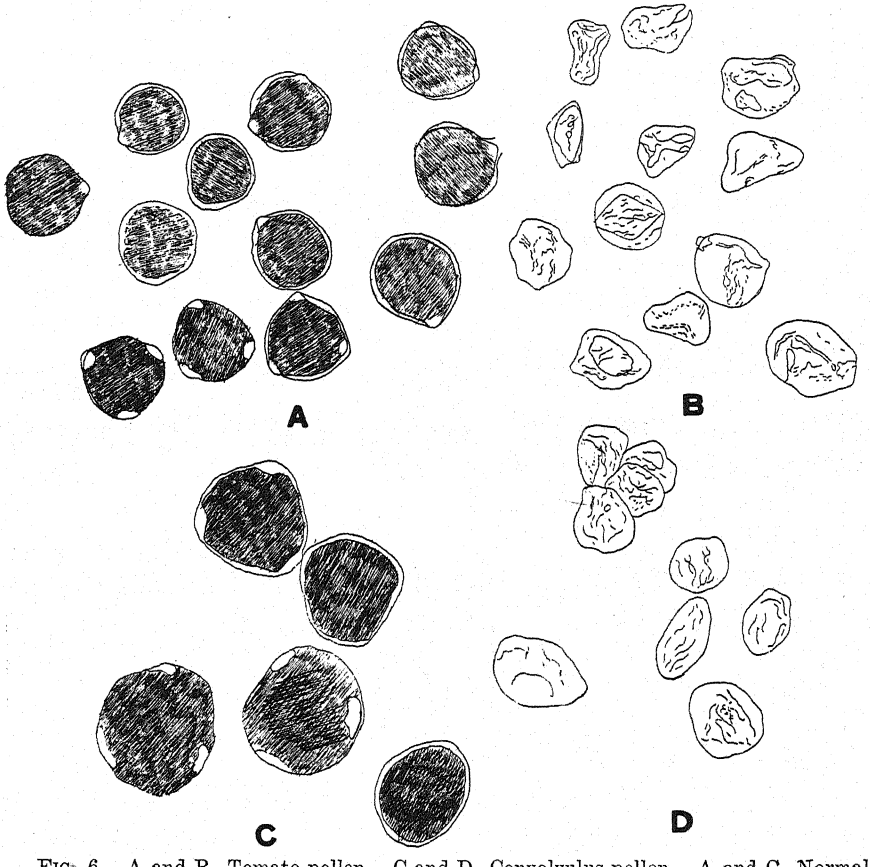


FIG. 6. A and B. Tomato pollen. C and D. Convolvulus pollen. A and C. Normal. B and D. *Stowboor.*

Microscopic examination of a fruit shows the starch content greatly changed in comparison with the above-mentioned parts of the plant. While diseased plants have little or no starch, the cells of healthy plants are filled with starch. While the fruit is yet quite young, there is no difference in its starch content, but when it has grown and measures 2 cm. or more in diameter we find that some of the cells of the diseased plant have very small starch grains and that these are present only in a greatly restricted number, while healthy fruits are rich in starch.

In examining a cross section through the fibrovascular bundles of a diseased tomato, it may easily be seen that lignification has affected the vessels only. There are 4 to 5 bundles in each fibrovascular tract (Fig. 1, A) in a normal fruit. A diseased fruit presents no such phenomenon. Besides the vessels, the lignification also extends to the other elements of the xylem,

disposed in the shape of a horseshoe or more or less in a circle. (Fig. 7, C). With a view to determining the constituents of the xylem in a more exact manner, the tissue of a healthy as well as of a diseased fruit was macerated in Schultze's solution.

The macerated tissue of a healthy fruit was mostly formed of vessels with spiral, thickened walls, of tracheids, and a few libriform cells (Fig. 4, D and 7, B). The tissue of diseased fruits consisted almost entirely of porous vessels that presented the appearance of shortened and greatly thickened cells. Spiral vessels in the macerated tissue of a diseased fruit were very scarce, but this tissue contained libriform cells in abundance. (Fig. 4, E and 7, A).

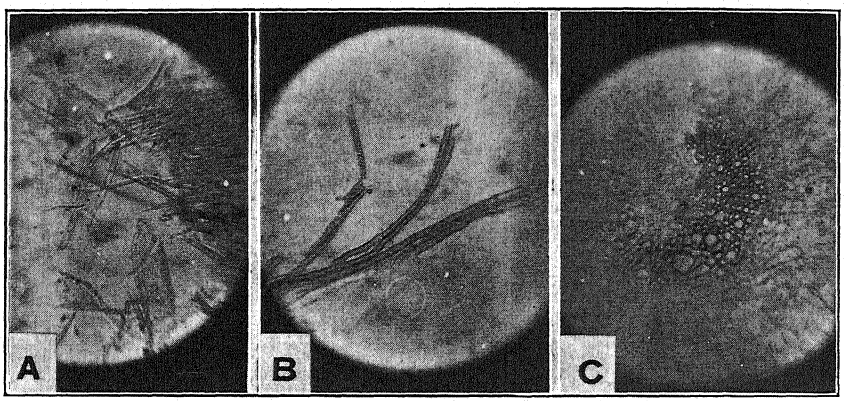


FIG. 7. A and B. Macerated tissue of tomato fruit. A. Diseased. B. Normal. C. Fibrovascular bundle of a *Stowboor* tomato fruit.

In the foregoing we have noted the pathological condition afforded by all the parts of a diseased plant. The separate parts of such a plant develop faster than those of a healthy individual. In the former we find completely developed fibrovascular bundles at an earlier stage of development than in a normal plant; the same may be said of secondary wood formation, and of the appearance of bast (in the stalk and pedicels). In the xylem we find almost no spiral vessels, proof of an earlier stage of development of the plant, whereas in a diseased plant all the spiral vessels become dotted or scalariform. In one instance a spiral vessel was observed even to turn into a porous one. Once we observed a vessel part of which had spiral thickenings while another part was porous (Fig. 4, C). These findings show that spiral vessels may change into porous ones.

The influence exerted by the virus on the inner phloem is especially evident; it is strongly developed in diseased plants and the phloem-cells are filled with a yellow substance, the nature of which, unfortunately, could

not be determined. This material may hinder the regular evacuation of organic substances, and, as a result, all of the parenchymatous cells of the stalk, the peduncle and the pedicel are crowded with grains of starch, while the fruits are quite void of starch or contain but little of it. It is interesting to compare these findings with the data recorded in the literature on pathological-anatomical changes that result from viroses. The filling of tissues with starch has been observed in different viroses. The monograph of V. Rischkov (3) summarizes the literature on this problem.

Here we need only to point out that in this instance the crowding of the tissues with starch was not accompanied by necrosis. This is definite proof of the fact that the conductivity of phloem may suffer even without any such gross changes. Probably the peculiar alteration of the contents of the sieve tubes, previously noted, is the outward expression of the loss of normal conductivity by the phloem.

Rochlina (5) noticed that potatoes affected with virus-diseases age promptly, developing sclerosis at an early date; but in none of the reported instances was this so strongly marked as in those reported by the writer. Of all the data recorded in the literature examined by the writer, the phenomenon as disclosed by the studies here presented, strongly recalls the changes in Passiflora caused by woodiness. In studies reported by others, hardening and tastelessness of the fruits are also characteristic of the disease, but in such cases woodiness is caused by excessive sclerosis of the peripheral layers of the pericarp parenchyma (2), while in the writer's case it is caused by peculiar alterations in the bundles.

The changes here described are unknown in other viroses and are specific for woodiness of tomato fruit. The symptom, therefore, possesses diagnostic value. Since the elements of xylem retain their characteristic structure, even in macerated fruits, the disease may be diagnosed even in mashed fruits or in those forming part of various dishes. The diagnosis is established on an anatomical basis. Unfortunately, Samuel, Bald, and Eardley (6) give only a short description of the anatomical alterations in tomatoes suffering from big bud. Still, their description proves that the morphological features of big bud and fruit hardening are just as identical to each other as are the anatomical symptoms. The Australian investigators have, like myself, observed hypertrophy of the inner phloem and crowding with starch grains in the tissues of diseased plants.

Although Samuel, Bald, and Eardley (6) do not mention any of the changes that I have observed in the xylem of the fruit, one may hardly doubt that they also occur in cases of big bud, for this disease is likewise characterized by a hardening of the fruit.

The study of anatomical symptoms of woodiness of tomato fruit and big bud has persuaded the writer that these diseases are identical.

In discussing the peculiar anomalies of histogenetic character observed in cases of woodiness we must, first of all, consider whether or not these changes in the fibrovascular bundles depend on a certain reduction in the size of the leaf blades of diseased plants. It is known that the kind of correlation existing between the leaf and the fibrovascular system of the stalk has been determined by experiment (10), as well as by observations (1). Zhakaja (10) noted the different changes that occur after the removal of elements of the node. Of these changes the increased development of the xylem is of special interest here. The changes of the leaf blade noted in woodiness of fruit are, however, comparatively unimportant and not comparable, for instance, to the filiform leaves seen in some mosaics of tomatoes, notwithstanding the fact that in the latter no profound alterations seemed to have taken place in the fibrovascular bundles. We, therefore, are bound rather to explain the anomalies of the xylem and the phloem by a specific action of the virus than by changes in the node elements.

PATHOLOGICAL-ANATOMICAL CHANGES IN WOODINESS OF FRUIT IN BINDWEED, CONVOLVULUS ARVENSIS

On tomato plantations and in their neighborhood there grows a weed named *Convolvulus arvensis* or bindweed. Very often it is attacked by a disease closely resembling woodiness of fruit (*Stowboor*). The diseased plants bear small, round leaves. Just as in the case of diseased tomato plants, their leaves are chlorotic, especially at their margins, which often curl upwards. A diseased bindweed seldom blooms and, when it does, its flowers show changes typical for stowboor. I have studied the anatomy of such plants.

THE ANATOMICAL STRUCTURE OF A BINDWEED FLOWER

In healthy individuals the superficial epidermis of the petals consists of one layer of cells, slightly drawn out in the direction of the breadth of the petal. The mesophyll has 9 to 10 layers with rounded, small, compactly disposed cells. Between the mesophyll and the epidermis there is a row of rounded cavities that probably extend along the whole length of the petal. The lower epidermis consists of a layer of cone-like cells with their apices directed outward. The fibrovascular bundles contain 2 to 3 vessels each and their phloem is hardly discernible (Fig. 5, F and G).

Cross sections of petals of a bindweed suffering from stowboor remind one of those of healthy petals. They are rounded and crescent-shape. Their mesophyll consists of roundish cells, lying close together. There are no such lacunae as we have seen in the petals of healthy plants. The size of the fibrovascular bundles in the center of the mesophyll is greater than in normal individuals, and the mesophyll cells are packed with chloroplasts.

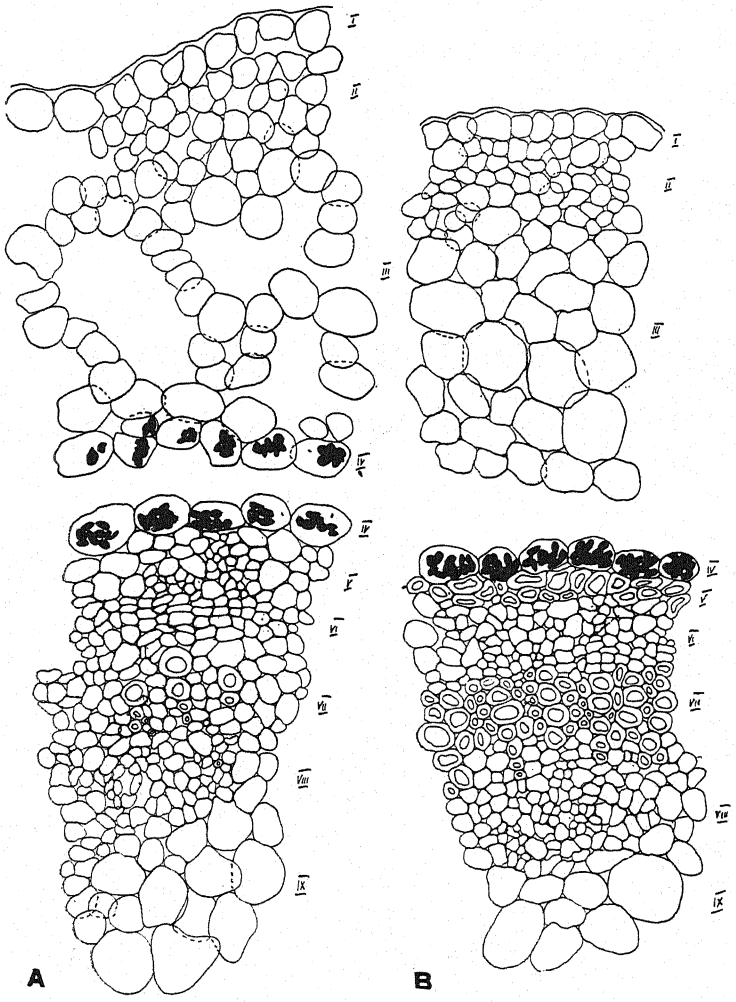


FIG. 8. A. and B. Cross section through peduncle of Convolvulus. A. normal. B. *Stowboor*. In both A and B: I, epidermis; II, assimilating tissue; III, cortical parenchyma; IV, bundle sheath; VII, xylem; VIII, inner phloem; IX, pith. In A: V, outer phloem; VI, cambium. In B: V, bast; VI, outer phloem.

The sepals of a healthy Convolvulus are crescent-shape in cross section. In the middle of the crescent the mesophyll consists of 13 to 14 layers of cells, while at the ends there are but 1 to 2 layers. The cells are rounded and lie close together. Nearer to the epidermis are lacunae of greater size than those of the petals. There are about 35 of them in a single section and they all lie in series. Next to the mesophyll there follow 3 to 4 cell layers whose walls are very thick; then comes an epidermis of rounded cells

forming a layer. The fibrovascular bundles are situated very close to these thick-wall cells. The bundles are poorly developed, having no more than 8 vessels each.

The cells of the upper and lower epidermises of the sepals of bindweed suffering from stowboor are quadrangular. Their outer walls are cutinized. The mesophyll consists of 6 compact layers of quadrangular cells filled with chloroplasts and starch. The midrib has 20 to 25 vessels surrounded by mechanical, nonlignified tissue and the phloem is poorly developed. The region of the midrib is swollen.

The midrib has 5 fibrovascular bundles, one of which is in the center with two smaller ones on each side (Fig. 5, K and L).

THE PEDUNCLE

The structure of the peduncle deserves special attention in a study of the bindweed, whether healthy or diseased. A cross section through a healthy peduncle shows an epidermis of a single layer of cells; 2 to 3 layers of assimilating tissue; a loosely formed parenchyma with large intercellular spaces; and 9 to 10 fibrovascular bundles forming a ring. The existence of a great many intercellular passages in the peduncle of Convolvulus is most interesting. They develop as they do in the stalk of water weeds. This likewise is characteristic of other kinds of Convolvulus, as, for instance, *C. cantabricus,* and of such other members of the Convolvulaceae, as Ipomoea. I have not been able to find in the literature the description of a like peduncular structure. Van Tieghem (9) does not mention it, although he gives a detailed description of the structure of the peduncle. In the systematic anatomy of Solereder (8) there also is no mention of it. Under the influence of virus the structure of the peduncle of a plant attacked by *stowboor* undergoes profound changes. The intercellular spaces of the cortical parenchyma utterly disappear. Thick bast develops and the xylem forms a continuous ring with numerous lignified cells of mechanical tissue. (Fig. 8).

The writer presents here the original record of the investigation.

Normal Plant

(1) Single layer of epidermal cells filled with protoplasm; 0.1395 mm. in diameter. Assimilatory tissue, consisting of 2 layers of cells, 0.1396 to 0.155 mm. thick, with chloroplasts and starch. The cortical parenchyma has enormous air-filled cavities; this parenchyma has a thickness of 1.1345 mm. to 0.93 mm.; inside the cells there is protoplasm and a few starch grains, visible after treatment with iodine. The cells of the one-layer bundle sheath are crowded with starch grains; it measures 0.093 to 0.124 mm. In the middle of

Stowboor Plant

(1) Single layer of empty epidermal cells of 0.124 to 0.099 mm. in diameter. Assimilatory tissue, 2 to 3 layers, 0.124 to 0.155 mm., with chloroplasts. The cortical parenchyma is 0.5425 to 0.52 mm. broad; its cells are smaller than in the healthy plant and quite without any intercellular spaces. One layer of thick bast, measuring 0.0775 mm. The outer phloem is 0.217 to 0.2325 mm. broad. The xylem is continuous with a radius of 0.31 to 0.4135 mm. The inner phloem is 0.275 to 0.341 mm., large, continuous; sieve tubes plainly visi-

the peduncle (2.728 to 2.371 mm.) are large cordate cells with wide intercellular spaces; the cells contain protoplasm and several grains of starch. The core is surrounded by inner and outer phloem and xylem. There are 1 to 2 areas of xylem with 3 to 4 vessels each. Most of the latter are spiral-shape; also, there are 5 areas with 10 to 13 spiral vessels. No more than 3 vessels are met with in a xylem tract.

ble. The core measures 0.93 to 1.24 mm. The cells are big, without contents; intercellular spaces small. No starch.

The investigation of bindweed affected with *stowboor* proves that (1) Under the influence of the virus the peduncle becomes much hypertrophied. (2) The xylem develops more intensely than in a healthy Convolvulus plant and forms a continuous ring in the peduncle. (3) The aboveground parts of the plants contained more starch than was present in healthy ones. (4) The leaf mesophyll is without intercellular spaces. (5) The pollen of a Convolvulus plant affected with *stowboor* is sterile. (Fig. 6, C and D). The above-mentioned symptoms are characteristic also for woodiness of tomatoes. Naturally, only artificial infection of tomato plants with *stowboor* from a diseased bindweed would definitely prove the identity of both diseases. In conclusion, I am much indebted to Prof. Dr. V. L. Rischkow for his very helpful suggestions and advice.

SUMMARY

The writer has studied the anatomical structure of all the parts of tomatoes affected with woodiness of fruit, as well as the flowers and peduncles of a Convolvulus suffering from a disease of the same type.

In the tomato the following facts were observed:

The stalks of a tomato plant affected with woodiness of fruit develop faster than those of a healthy plant. The interfascicular wood and the ring of bast appear earlier than in the healthy plant.

All the tissues of the aboveground parts undergo a more or less pronounced hypertrophy.

Hypertrophy is especially well expressed in the inner phloem, which occupies, in cross section, an area equal to that of the outer phloem and xylem taken together.

The cells of the inner phloem are filled with a pale yellow substance.

The vegetative parts of a tomato are filled with starch grains; the leaf blade forms an exception to this rule. In the fruits, starch disappears very early, contrary to the condition observed in healthy plants.

In cases of woodiness of fruit, the structure of the mesophyll of the leaves and petals shows almost no intercellular passages in the mesophyll of diseased tomatoes.

The thickness of the peduncle and the pedicel of diseased tomato plants, as well as the degree of development of the mechanical tissues in the same, exceed those of a healthy plant, although the fruits are smaller.

In the upper parts of the peduncle and pedicel and in the anomalously lengthened axis of the flower, we observe a polystelic structure.

The pollen of the flowers of plants suffering from woodiness is sterile.

In diseased fruits the fibrovascular bundles are strongly lignified.

The wood of these bundles contains much libriform tissue, which is lacking in xylem of healthy fruits. In diseased fruits, spiral vessels are replaced by porous vessels.

The changes in a diseased bindweed are the same as in a *stowboor* diseased tomato: reduced intercellular spaces, intense development of xylem, numerous starch grains in the tissue, etc.

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THE COTTON ROOT ROT FUNGUS, PHYMATOTRICHUM OMNIVORUM, PARASITIC ON THE WATERMELON, CITRULLUS VULGARIS

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INTRODUCTION

Phymatotrichum omnivorum (Shear) Duggar, the destructive fungus that causes Texas root rot (cotton root rot), probably is the most important plant parasite in Arizona and the semiarid Southwest. The fungus has a known host range of more than 550 wild and cultivated species (22). The watermelon, *Citrullus vulgaris* Schrad., has been listed by Taubenhaus and Killough (25) as completely resistant to the fungus. Later Taubenhaus, Dana, and Wolff (21) list as immune or resistant species *C. vulgaris* and all other members of the Cucurbitaceae except the summer squash, *Cucurbita pepo* L., variety *condensa* (Bailey).

Repeatedly, infection of the watermelon with *Phymatotrichum omnivorum* has come to the notice of the Department of Plant Pathology of the University of Arizona. On June 29, 1930, S. L. Owens, County Agent of Yuma County, submitted specimens of dying vines from a field in which watermelons had followed alfalfa and were wilting in the spots where Texas root rot had killed alfalfa the previous year. From the dying vines *P. omnivorum* was obtained. The affected variety was not recorded. During the summer of 1932 two cases were reported. The first case came on July 7 from C. J. McCash, Assistant County Agent of Pima County. Later, M. M. Evans found watermelon plants affected with the disease. In both cases the variety of melon was Iowa King. Recently a 5-acre field of Klondike watermelons near Tucson was found to be badly spotted with Texas root rot. The infected area, totalling about two acres, was a total loss to the grower. A number of young peach and pecan trees planted in this field also died of root rot. Other cases have been reported from the Salt River Valley and the Casa Grande district. The reports stimulated an investigation to ascertain the degree to which watermelons are susceptible or resistant to Texas root rot; also an investigation of the penetration of the parasite and the effect of mixed cultures.

[1] The writer gratefully acknowledges his appreciation of the assistance given him by the following members of the Department of Plant Pathology of the University of Arizona: Dr. R. B. Streets, who supervised the investigations, Mr. M. M. Evans, and Dr. J. G. Brown.

RESISTANCE

General. Although the breeding and selection of plants resistant to or immune from disease is the ideal method of plant-disease control, little is known about the absolute cause or basis of true resistance in plants. Different plants vary greatly in their resistance to disease. One or more factors may enter into a given case of resistance. The factors may be exceedingly complex, involving a detailed study of morphological systems and physiological processes. Resistance in one case may be due to the presence of a substance in the host that is toxic to the parasite; in another case it may be caused by an entirely different factor. Obviously, practically every problem of resistance in plants is necessarily a specific one and must be attacked as such.

Resistance of Plants to Phymatotrichum omnivorum. Although Texas root rot has been investigated since 1888 (16), comparatively few resistant plants have been found. Since the fungus is indigenous to the Southwest (10) and its sclerotia, which may remain viable for several years, are present in infested soils (15), the problem of resistance obviously is important. Recently it has been reported (13) that strands of the organism remain viable and infectious in certain soils for 3 or more years. This discovery adds to the complexity of the problem.

The literature presents a limited amount of information on the nature of the resistance of plants to Texas root rot. However, many workers have studied in detail various phases of the disease and their contributions are valuable. As early as 1888 Pammel (16) advocated the rotation of crops on diseased soil. In his original paper on root rot he said: "Corn, sorghum, millet, wheat, oats, and other members of the grass family do not die from this cause, and consequently should be used after cotton." In 1907 Shear (18) (19), with field experiments, showed the value of proper crop rotations. With a 2-year rotation of corn and wheat, he obtained a decrease of 95 per cent of the cotton dying from root rot to 5 per cent after the rotation. Similar recommendations are given by McNamara (12).

There has been some difference of opinion as to the degree of resistance or immunity of various monocotyledonous plants. King and Loomis (11) reported strands of *Phymatotrichum omnivorum* on roots of the date palm and on grass roots, but did not prove that the fungus gets nourishment from such roots. On the other hand, Taubenhaus and Ezekiel (23) (24) showed rather conclusively that monocotyledons are very resistant to the fungus. They reported that, although date palms, corn, sorghum, Johnson grass, Bermuda grass, and other grasses were interplanted with cotton and all plants were inoculated, only the cotton plants died. Depressed, deep brown lesions were found on roots of some monocotyledonous plants, but isolations from these failed to yield *P. omnivorum*.

Studies on the physiological basis of resistance have been published by Ezekiel, Taubenhaus, and Fudge (7). They show that cultures of *Phymatotrichum omnivorum* grow much more luxuriently on cotton media than on corn media. The heaviest growth of the fungus was found when the largest supply of dextrose was available. In a later report by the same authors (8) very slight fungus growth was obtained on undiluted juices of monocotyledons (corn, onions, cannas, and nut grass), although heavy growth was evident on diluted juices of susceptible dicotyledons (cotton, carrots, turnips, and sweet potatoes). Furthermore, upon dilution of the juices from monocotyledons the growth of the parasite was not inhibited. The experiments are interpreted to indicate that monocotyledonous plants contain a substance or substances that, in sufficiently high concentrations, can inhibit growth of the root-rot fungus, and that the resistance of the plants is based, at least in part, upon the presence of the substance or substances.

More recently, Moore (14) has shown that corn, wheat, and barley media do inhibit growth of *Phymatotrichum omnivorum*. She states that the inhibition is due to the presence of labile, water-soluble substances in the corn roots, and that these substances in small quantities may act as stimulants.

It is interesting to note that the Turk's-cap hibiscus, *Malvaviscus conzattii* Greenman, is the only plant out of 82 species belonging to the family *Malvaceae* that has shown no natural infection with the root-rot fungus under field conditions. Some 1-year-old plants became infected following inoculation, but they recovered. The factors involved and the nature of this resistance have not yet been revealed (1). Moore (14) believes that in this case resistance may be due to the presence of labile substances similar to those present in monocotyledonous plants.

Certain other plants, such as the hackberry, live oak, pomegranate, and many others (21), are known to be highly resistant to root rot, but the nature of the resistance is still obscure. For several years, in Texas (22, 6) as well as in Arizona, a large number of varieties of cotton and selections of individual strains have been tested for possible resistance to root rot. Many of these plants have been eliminated because of susceptibility, but the remaining ones are still under test. Nevertheless, no similar work has been published in regard to the watermelon plant.

EXPERIMENTAL WORK

Materials. The watermelon hosts used in the studies here reported consisted of the wilt-resistant Iowa King, which is becoming a popular variety in Arizona; the wilt-resistant varieties, Iowa Belle and Pride of Muscatine; and the wilt-susceptible Black-seeded Klondike. With the exception of

Black-seeded Klondike, the plants were grown from seed originally obtained from the Iowa Melon Growers' Association. Acala cotton seedlings, grown from seed supplied by R. B. Streets, of the University of Arizona, also were studied to a limited extent because of their known susceptibility to the Texas root-rot fungus.

As a source of inoculum, sclerotia from Goodyear and Sacaton, Arizona, were obtained from C. J. King, of the United States Field Station at Sacaton. Several sclerotia were surface-sterilized 3 minutes with a 1–1,000 mercuric chloride solution placed on sterile filter paper and transferred to potato-dextrose-agar slants. No growth was evident until the third day, and then it was very slow and scant. It was not uncommon to find a mixed culture growing from a single, surface-sterilized sclerotium. The pure culture from a single sclerotium was increased on steamed carrots, plugs of watermelon rind, sweet potatoes, potato-2 per cent dextrose agar, and other media.

Methods and Results. The field experiments were conducted at the New University Farm near Tucson, at an elevation of 2300 feet. The soil is a light sandy loam with a moisture equivalent of 12 to 15; the alkalinity is variable, although the differences between the first and second feet in any one case were not great; the average pH was found to be 8.48 with a minimum and maximum of 8.3 and 8.65, respectively. No temperature records are available for the plot studied, but air and soil temperatures recorded on the campus are given below (Table 1). Over a period of several years the air temperatures prevailing on the campus are known to average 4 or 5 degrees F. higher than those prevailing at the Farm.

TABLE 1.—*Air and soil temperatures, University of Arizona campus, Tucson, during period of field inoculations*

	Air temperatures (U. of A. Weather Bureau)							
	1932				1933			
	Aug.	Sept.	Oct.	Nov.	May	June	July	Aug.
Max.	105	103	93	84	103	111	106	.....
Min.	64	57	36	32	38	52	67	.....
Mean	85.2	80.6	67.8	60	67.5	80.8	87.7	.....
	Soil temperatures: Mean temperatures at depth of 4 inches based on weekly averages (recorded by M. M. Evans)							
Max.	82	83	67.2	61	69	84	83.2	.....
Min.	76	74	62	54.5	62	72	77	.....
Mean	79	78.6	64.6	57.75	65.5	78	80.1	.....

Cotton had been grown on the plot for several years and active root-rot spots were present. Considerable damage occurs annually in adjoining

fields of alfalfa and cotton. No sclerotia of *Phymatotrichum omnivorum* have been found in the field in which the plot was located.

On September 30, 1932, 40 days from date of planting, 39 watermelon plants were inoculated by placing infected cotton roots in the soil in the center of each hill. Eighteen plants were Iowa Belle and 21 were Pride of Muscatine. Two large plants of the Muscatine variety were carefully excavated on October 22 and the roots were thoroughly examined, but no characteristic strands or lesions were found. Six days later the roots of 2 Iowa Belle plants were examined with similar result. On November 5 one large plant of each variety was examined and found healthy. Fourteen days later (50 days after inoculation) the plants remaining in the plot were removed

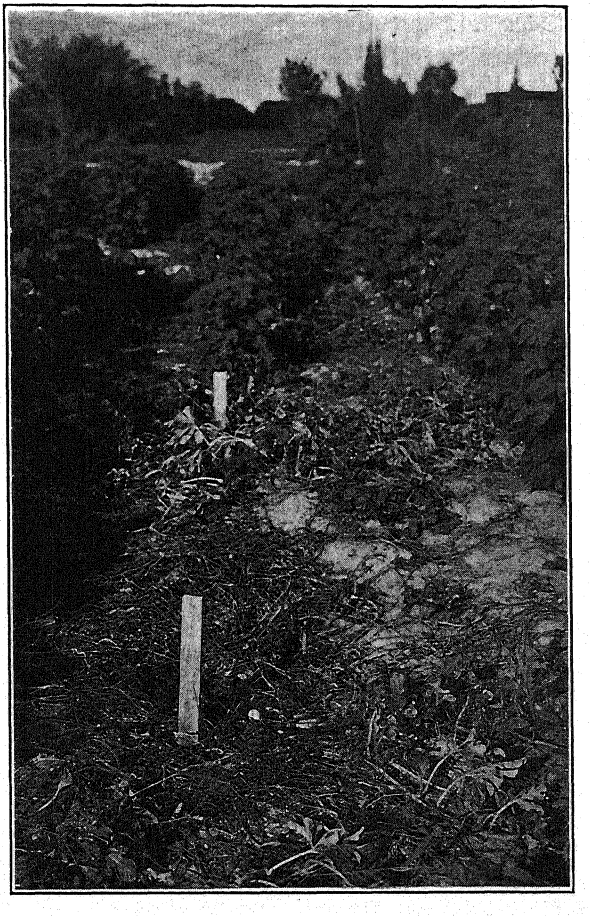


FIG. 1. Watermelon vine killed by *Phymatotrichum omnivorum* after inoculation with a pure culture of the fungus.

3 days after a killing frost. Precautions were taken to obtain as complete root systems as possible. Examination in the laboratory disclosed typical lesions with connecting strands on roots of both varieties of watermelons. Portions of infected roots were prepared for cytological study and other pieces were cultured. Attempts to isolate the fungus from this material were not successful.

On the same soil watermelon seeds of Iowa Belle, Iowa King, and Black-seeded Klondike were planted in hills about 2 feet in diameter on April 28, 1933. All hills except 2 of each variety were inoculated on June 19. The inoculum was a pure culture of *Phymatotrichum omnivorum* grown on steamed carrots in 750 cc. flasks for 39 days. The inoculum was removed from a flask and placed in a hole approximately 6 inches deep in the center of each hill. At this time alfalfa plants in the adjoining field were wilting from root rot. Up to and including July 7, 18 days after inoculation, there was no evidence above ground that disease was present. On this date several plants were carefully removed and examined. Strands and small lesions were found on the roots of 3 plants. Pieces of roots with lesions were cultured in the laboratory and *P. omnivorum* was isolated from 2 cultures.

On October 26 (about the end of the growing season) all of the plants in this experiment were dead or dying from root rot except one of the check plants. The other check plants had by this time contracted the disease from adjacent inoculated plants. Of a total of 36 plants, 35 became infected with root rot.

In the previously described experiments the watermelon plants were artificially inoculated; the disease also occurred naturally in the field. On the watermelon, like most other hosts, the disease results in a sudden wilt-

PLATE I

A. a. Iowa King pure culture seedling 25 days old. Dead from attack of *Phymatotrichum omnivorum*. Note the brown roots with fungus. ×1.5. b. Check; white clean roots against tube. B. Lateral root from Black-seeded Klondike in field. Note lesion and strands. ×1.5. C. Iowa King seedlings on agar slants. In a and b Phymatotrichum has surrounded roots of seedlings and is growing on them; Ck shows vigorous sterile conditions of check. ×1. D. a. Pure culture of Phymatotrichum on 2 per cent potato-dextrose agar. b. Inhibiting effect of *Trichoderma lignorum* on *P. omnivorum*. 7 days from date inoculated. a and b inoculated at same time. ×.5. E. The root system of a diseased watermelon plant of the variety Klondike. ×.15. F–H. Photomicrographs of watermelon roots infected with *P. omnivorum*. F. Shows that the fungus filaments are largely intercellular. From root on agar culture. 5 days. Cortical tissue. ×400. G. Wedging of fungus on outside of large root from field, Black-seeded Klondike variety. Section 2 inches below soil line. ×25. H. Cross section of root 48 hours after inoculation. Iowa King variety. ×10. Photographs A and C by R. B. Streets; all others by M. M. Evans.

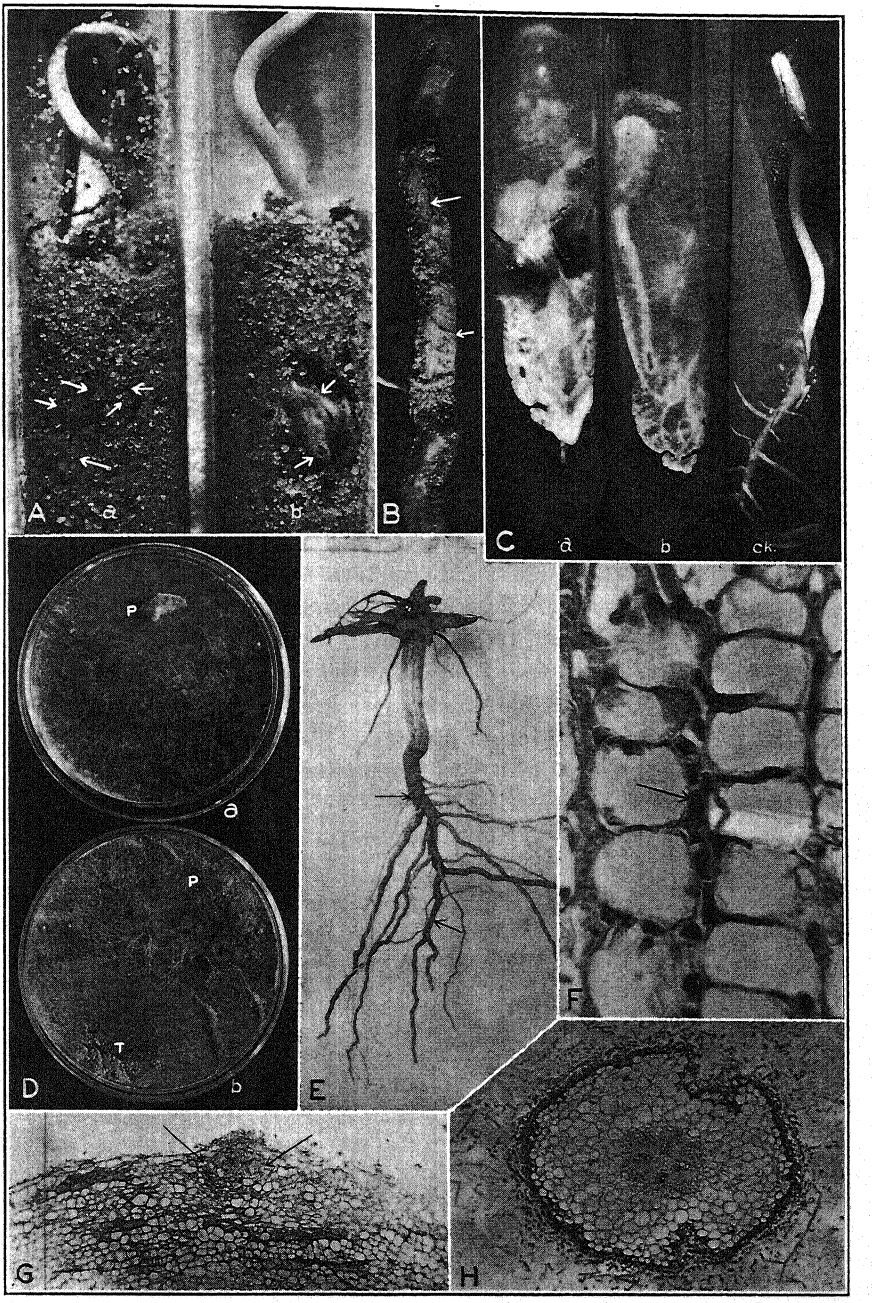


PLATE I

ing and drooping of the leaves. On July 11 one plant of the Klondike variety wilted (Fig. 1). The affected plant in this case was somewhat pale yellow. The root system was removed from the soil to the laboratory, together with that of an unwilted plant from the same hill.

When examined the root system of the wilted plant showed late stages of infection with the Texas root rot fungus (Plate I, E). The taproot was dirty brown and the rotted condition was more advanced toward the tip, indicating that early infection probably had taken place at least 12 to 14 inches below the surface of the soil. Typical, brownish strands of the fungus were prevalent on the surface of the taproot and lateral roots. On the first 2 inches of the taproot there was no external evidence of infection, but at 3 inches there were slight signs. Some parts of the root system were so completely rotted that the soft, slimy cortex could be easily removed from the woody central cylinder.

Very few lateral roots were present on this specimen, although the rotted remains of many were found in the soil. There were more lateral roots on diseased plants of Black-seeded Klondike (Plate I, B). Infection was generally more severe on the lateral roots near their attachment to the main root than farther out. This may indicate that infection had spread from the main root to the laterals, although the opposite condition was sometimes found. Different parts of the roots were killed in Gilson's reagent for further study.

The excavated plant, which bore no visible symptoms of disease above ground, was found to have brown strands of the Phymatotrichum fungus on its roots; a few small, light brown lesions were present.

PLATE II

A. A single hypha growing between two epidermal cells. Root of Iowa King in contact with inoculum 48 hours. B. Penetrating hypha between two cells then into a cell and in contact with the host cell nucleus. Note constriction of hypha at point of entry. C. A later stage showing the development of the hypha in the host cell in direct association with the cytoplasm and the exceedingly small host nucleus (*n*). Also note the cross section of hypha growing intercellularly. D. Hyphae wedging and pushing into root. Note multinucleate fungus cells; 4 nuclei in one case. E. The host cell wall has been stretched and, although the fungus is exerting considerable pressure, penetration has not yet been accomplished. F. Penetration has been accomplished just as the hypha had begun development intercellularly. G. Direct penetration has taken place and the hypha has branched into an haustorium-like body. Note the accumulation of mycelium on outside of host cell; also the direct contact of host cell cytoplasm and fungous hyphae. H. Cortical parenchyma cell isolated by enveloping hyphae, 5 days from time inoculated. Note the shrinking of the protoplasm from the cell wall. I. Coiling of *Trichoderma lignorum* filament (*t*), around *Phymatotrichum omnivorum* filament (*p*). Note vacuolate condition of the root-rot-fungus. J. *Trichoderma lignorum* filament inside of root-rot aerial hypha. The Phymatotrichum filament is without protoplasm when in association with Trichoderma. All × 853.

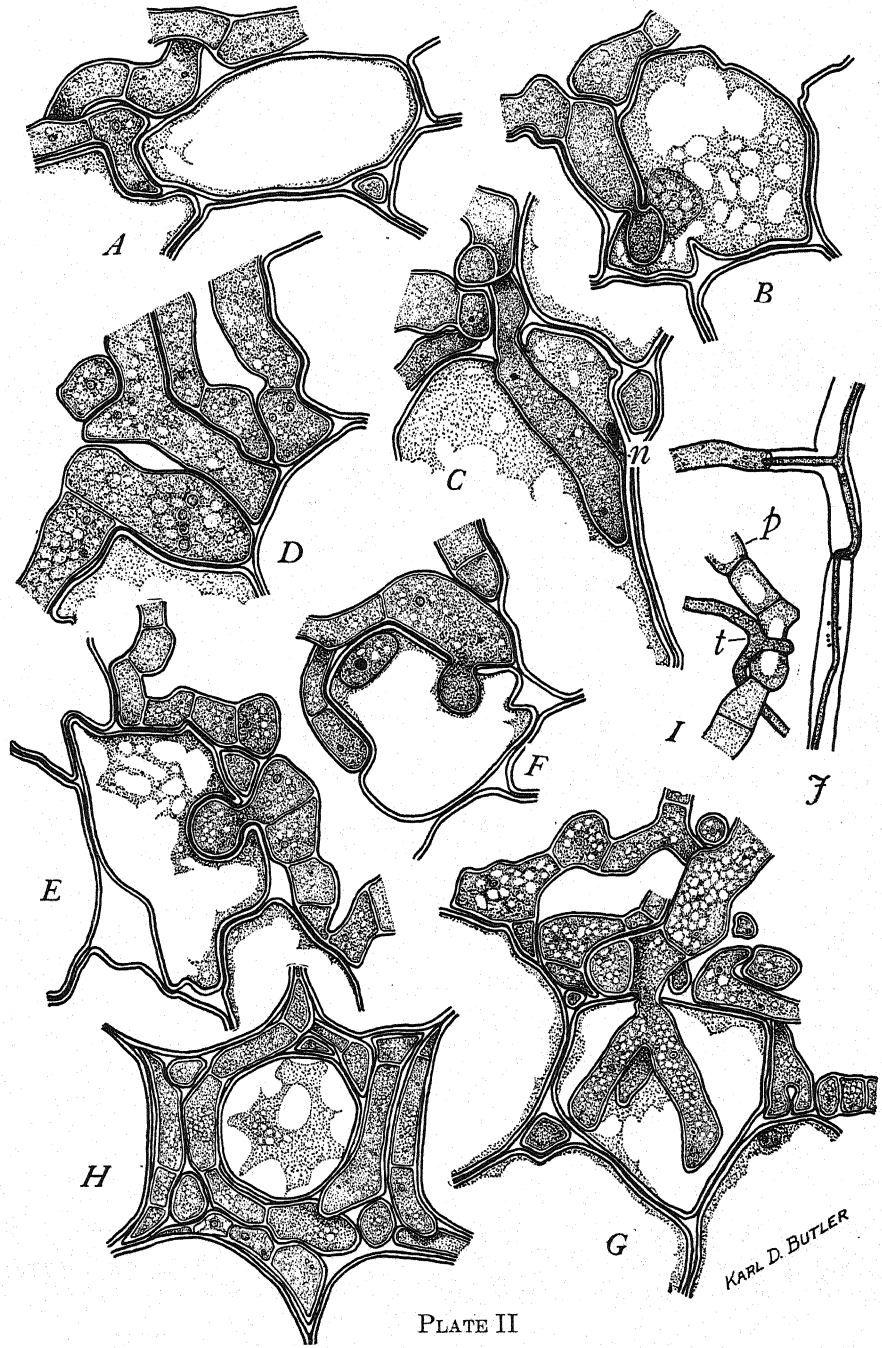


PLATE II

On the stained sections made from this material 2 inches below the soil line, the wedging, mass action of the penetrating fungus was evident at many places (Plate II). The parasite had advanced through and between only 6 to 8 rows of cortical cells, but the mycelium was rather uniformly distributed in the infected area. Fungus filaments were prevalent in the vessels in the more severely attacked parts of the roots, and the cortical tissue was collapsed and dead.

In general, it appears that the cortical cells nearest the invaded cells contain less protoplasm than do those farther removed; also, cells penetrated with hyphae often show plasmolysis (Plate III, F). In some cases this had begun where a hypha was in contact only with the outside of the cell. A marked difference was noted between the nuclei of invaded and noninvaded cells. Those in invaded cells were distinctly smaller, stained much more deeply, and appeared to be in a state of inactivity (Plate III, C and F). The nuclei in noninvaded cells not only were larger but also clearly showed the usual fine nucleoplasmic network and contained a prominent nucleolus (Plate III, E). Measurements of greatest nuclear diameters gave the following results: in the noninvaded cells the maximum diameter was 14 μ, the minimum, 8 μ, and the average 11.8 μ; in invaded cells these diameters were 6.5 μ, 3 μ, and 4.8 μ, respectively. Only nuclei in noninvaded cells 3 or less cells distant from the invaded ones were measured. The nuclei of invaded cells averaged less than half the diameter of those of noninvaded cells. The manner of fungal invasion of the cells was investigated in the laboratory.

Penetration Studies. Detailed cytological studies on penetration by various fungi have been discussed (20, 2, 4). There appears, however, to be no report at hand of any similar study of the Texas root-rot fungus. The early penetration stages of the fungus having never been figured or described, they are presented here. The most complete description of the penetration of the parasite is that given by Peltier, King, and Samson (17).

PLATE III

A. Fungus hyphae passing from one cell to another in cortex. Invasion from both intra- (*a*) and intercellular (*b*) spaces. Note scant protoplasm in host cell. Two hyphae entering through same hole in cell wall. B. Branched filaments in a lateral root growing between parenchymatous cells toward the main root. 5 days from time inoculated. C. An invaded cortical cell in a mature watermelon root from the field. Smaller and denser nucleus than in an uninvaded cell (E). Filament in direct contact with vacuolate host protoplasm. Also a fungus nucleus appears to be in the process of division. D. A branched filament in xylem cells of a 25-day-old plant. E. A noninvaded cortical host cell only 3 cells from invaded tissue. Note the large nucleus with the fine nucleoplasmic network. F. A cortical cell permeated with fungus filaments. Note the extremely small host nucleus, the shrinking of protoplasm from the cell wall and the scant protoplasm. B, E, F are from field material. ×853.

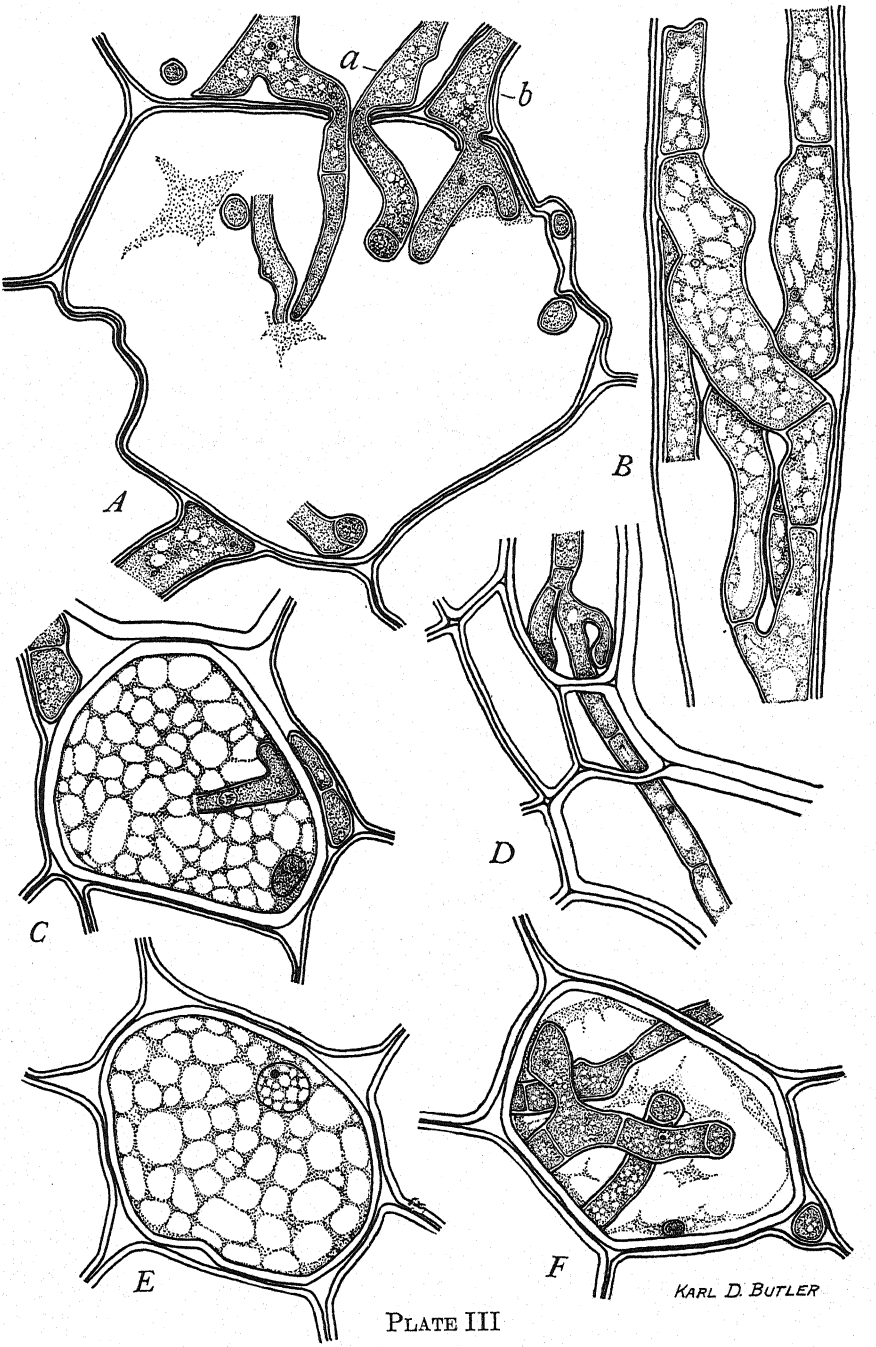


PLATE III

In their work, however, they did not use pure cultures and the field was covered only in a preliminary way.

Penetration of the fungus into the watermelon plant was studied with seedlings grown on sterile filter paper and potato-2 per cent dextrose-agar. Seeds of Iowa King were surface-sterilized by a 3-minute immersion in 1:1,000 mercuric chloride solution, rinsed thoroughly in sterile distilled water, and arranged on wet filter paper in sterile Petri dishes so that all roots could grow in the same direction. The dishes were now placed in a vertical position in an incubator at a constant temperature of 28° C. At the end of 48 hours the seeds that had germinated were transferred with flamed forceps to potato-2 per cent dextrose-agar plates, inoculated with *Phymatotrichum omnivorum* 4 days earlier. The inoculum was a 15-day pure culture on steamed carrots. The plants were placed vertically with the roots in the same direction. The roots varied from 5 to 10 mm. in length.

The cultures, divided into 5 groups, were observed under the binocular microscope at intervals of 8 hours. Group 1 was removed from the incubator after 24 hours; groups 2, 3, 4, and 5 at the end of each succeeding 24-hour period, respectively. Upon removal the roots of the seedlings were placed in Gilson's fluid and maintained 12 hours at 70° C. The material was then washed, dehydrated, cleared, embedded in paraffin, sectioned 5 to 10 μ thick, and stained. Heidenhain's iron-alum haematoxylin proved very satisfactory, especially when sections were counterstained with safranin.

Examination of the seedlings with the binocular microscope showed that the large-cell type, and more often the young strand types of hyphae (so designated by Duggar (3)), had started to grow around and on the roots after 8 to 16 hours. After 24 hours the entire root often was surrounded by the fungus, especially near the root-tip. No actual penetrations were observed with sections of 24-hour cultures. Nevertheless, some wedging action had begun.

The following 3 modes of fungal invasion of the young seedlings were noted: (a) by direct penetration into a cell, (b) by growing between two epidermal cells and finally into a cell, and (c) by the wedging of several or many hyphae, thus causing a lesion.

The roots of seedlings that had been in contact with the culture of the fungus for 48 hours were completely surrounded by mycelium (Plate I, H). The cells of the hyphae were rich in protoplasm, usually large, of irregular shape, with rather thick walls, and multinucleate. It was not uncommon to find 3, 4, or more nuclei between septa (Plate II, D).

Actual penetration had taken place in many instances after 48 hours, (Plate I, H). Although direct entrance into an outer cell was not unusual,

it was less common than intercellular penetration (Plate I, G, and Plate II, A, D). In either case it was evident that pressure was necessary to initiate final penetration. Often a hypha progressed between the cells and finally entered. Apparently, a softening action took place at the point of entry in addition to the mechanical pressure (Plate II, E). In no case was there evidence of any tearing or irregular rupture of the penetrated wall. The portal of entry was smooth and nearly always smaller in diameter than the invading hypha; consequently, that part of the hypha that passed through the host cell wall was usually smaller than at either side of the wall (Plate II, B, and Plate III, A). There were many separate and distinct invasions.

The fungus invades living cells. The cells of host plants grown in the manner described were somewhat vacuolate, yet quantities of protoplasm were not uncommon in invaded cells and even in contact with the hyphae (Plate II, B and C). Although haustorium-like bodies were noted and figured (Plate II, G), no true haustoria were observed.

After the initial hyphal entry, the fungus cells often were slightly smaller, of irregular shape, with very dense protoplasm containing few small vacuoles, and sometimes the septa were numerous (Plate II, E–G). It is, however, difficult to make any generalization, for the fungus was varied and irregular within the host tissues.

The spread of the fungus in the host usually occurred between the cells (Plate I, F, and III, B) with no visible evidence of cell-wall disintegration except at the place of entry. The hyphae branched freely and sometimes even completely surrounded a cell or cells (Plate II, H).

The fungus also advanced from cell to cell by passing directly through the cell walls (Plate III, A). In most cases the hyphae were constricted where they passed through the wall. Since these observations were made on young seedlings and the entrance of the fungus was in a small area just above the root cap, the spread was largely upward toward the stem (Plate III, B). In sections of roots of older infected seedlings grown in soil cultures the fungus appeared to spread in all directions from the place of entry. It also was found in the xylem of these roots, where it had advanced from one cell to another (Plate III, D). In no case in the seedlings grown on agar was there any visible differences noted between invaded and noninvaded cells.

Watermelon seeds (Iowa King variety) were surface sterilized by a 3-minute immersion in a 1:1,000 mercuric chloride solution. The seeds were then rinsed in sterile distilled water. Each seed was dropped with flamed forceps into a separate test-tube slant of potato-2 per cent dextrose-agar and placed in the incubator at a constant temperature of 28° C.. In 3 days all of the tubes were inoculated with the root-rot fungus except 4,

which were used as controls. All tubes were now kept on top of the incubator in the light in order to reduce etiolation as much as possible. The pure-culture inoculum was applied as a pseudo-sclerotium from a culture or as a piece of medium containing the fungus.

Hyphae from the inoculum, upon making contact with a root or rootlet, were noted to grow along the latter or to coil around it, meanwhile branching profusely. Examples were photographed (Plate I, A and C) and killed in Gilson's fluid to be used in cytological study. Pure cultures were isolated from the infected stems and roots.

The preceding experiment was repeated on Black-seeded Klondike, with almost identical results, except that the fungus was apparently more virulent on these seedlings that were killed when they had made very little growth. A fungus, identified microscopically as *Phymatotricum omnivorum,* was isolated repeatedly from the roots and stems of the seedlings.

The fungus was even more rapid and severe in its attack on Acala cotton seedlings grown in the same manner. Hyphae enveloped the roots very rapidly, then advanced up the stems onto the cotyledons, as was also noted in a few cases on the watermelon seedlings.

Eight-inch test tubes 1 inch in diameter were filled with soil from a root-rot area at the University farm near Tucson, Arizona. Water was added, the soil adjusted to water-holding capacity, and the tubes autoclaved for 2 hours at 15 pounds' pressure. Upon cooling, all tubes except controls were inoculated by placing a pure culture of *Phymatotrichum omnivorum,* grown on watermelon-rind plugs, underneath the surface of the soil. All tubes were placed in a constant-temperature incubator at 28° C. In 2 days mycelium was well distributed throughout the soil cultures, so watermelon seeds of Iowa King were surface-sterilized and planted, 2 in each tube. Prophylactic procedure obtained in all cases. Four days later, mycelium could be seen enveloping those rootlets growing against the inner surface of the tubes. The seedlings were now kept on top of the incubator where they received ample light. Sterile distilled water was applied at intervals to maintain the soil-moisture content. After 10 days the roots that could be observed where turning brown in places, and a binocular microscope showed them enveloped by abundant mycelium.

On the 25th day from planting 1 of the plants wilted and 6 more were beginning to wilt. The next day all 7 were dead. Seven days later all seedlings inoculated were dead, while the controls were in fine condition. Pure cultures of *Phymatotrichum omnivorum* were isolated from the diseased roots. Portions of the roots were placed in Gilson's fluid to be used in the study of the distribution of the parasite in the tissues.

A similar experiment was conducted, involving use of white sand instead of soil, and seedlings of both Iowa Belle and Iowa King were employed.

The results were variable. In general, the plants grown in soil cultures were most severely affected; in one case, however, large brownish lesions were found on the root of an Iowa Belle seedling 5 days after inoculum had been carefully placed near it. Iowa King seedlings, inoculated with a pure culture, were killed in 25 days (Plate I, A, a). Their roots had turned light brown and were enveloped with mycelium. The controls were in good condition (Plate I, A, b).

Effects of Mixed Cultures. In dealing with an aggressive and widespread soil-inhabiting parasite like *Phymatotrichum omnivorum,* its reactions in the presence of other fungi should be observed. From the standpoint of control the value of this information would be inestimable if some organism could be found that would definitely inhibit the growth and spread of the fungus without greatly impairing the normal growth of a host (9).

Neal (5) states that Aspergillus, Penicillium, and *Fusarium* spp. partially arrested the development of strands of *Phymatotrichum omnivorum* in agar plates simultaneously inoculated. Similar inhibiting qualities of the same fungi, as well as the depressing influences of various bacteria were noted in these studies.

In view of the work done by Weindling (26), a culture of *Trichoderma lignorum* was secured from Dr. H. S. Fawcett. Petri dishes of potato-2 per cent dextrose-agar, pH 7.2, were inoculated simultaneously on opposite sides with pure cultures of *Phymatotrichum omnivorum* and *T. lignorum.* The cultures were kept at a constant temperature of 28° C. Since the root-rot fungus grows more slowly than Trichoderma, the plates were inoculated with *P. omnivorum* on one side of the plate and after 3 days, when the fungus covered about one-third of the plate, the opposite side was inoculated with *T. lignorum.* The cultures grew directly toward each other without interruption. There was no evidence of any repelling influence of one fungus for the other. The fungi met in about 48 hours. Up to this time the mycelium of both cultures had grown very close to the culture medium, not projecting above its surface more than 3 mm. Where they contacted, some of the hyphae of each fungus grew straight toward the top of the Petri dish. At the same time most of the culture of *T. lignorum* grew over the top and in and around the root-rot fungus. On the other hand, the growth of *P. omnivorum* was almost completely stopped after the two cultures had encountered each other (Plate I, D, b). However, in a few cases the root-rot fungus grew luxuriantly along the roof of the Petri dish away from the mixture.

Wet mounds of aerial hyphae were examined at intervals. Several times a hypha of *Trichoderma lignorum* was found within a hypha of Phymatotrichum (Plate II, J). Evidently the former fungus obtains moisture and

nourishment from the root-rot fungus, for *P. omnivorum* was usually found to be very vacuolate and devoid of protoplasm when in association with Trichoderma. Furthermore, Trichoderma was found to twine and weave around strand hyphae of the root-rot fungus (Plate II, I).

To continue this investigation blocks approximately 1 cm. square of an 18-day-old culture on potato-2 per cent dextrose-agar permeated with both organisms were placed on agar slants. Precautions were taken to obtain blocks that were devoid of Trichoderma spores. In four days all tubes showed abundant growth of *T. lignorum,* but no growth of the root-rot fungus was evident. Even after 19 days *Phytomatotrichum omnivorum* had not grown from the inoculum. This indicates that the growth of the root-rot fungus is inhibited or killed by the presence of *T. lignorum.*

For an investigation of this nature to be of practical value, plants susceptible to root rot should be inoculated with a pure culture of Phymatotrichum and a duplicate set of plants with a mixture of the 2 fungi. This was done with Acala cotton and watermelon plants, but there are yet no results to report.

DISCUSSION

The data presented in these studies show that the watermelon, *Citrullus vulgaris,* is neither highly resistant nor immune, as was reported by Taubenhaus and Killough (25) and later by Taubenhaus and Ezekiel (24), but that it is susceptible. Field-grown plants of all 4 varieties, studied repeatedly, have been found infected with *Phymatotrichum omnivorum.* The different varieties vary in their susceptibility to the parasite and a detailed study of varietal susceptibility should be undertaken. In the field the roots of a few plants of the variety Iowa Belle apparently were infected by Phymatotrichum and the infected tissues were later walled off by the host, thus checking the spread of the fungus. In other cases the same variety was severely diseased. Thus it appears that the relative susceptibility may be variable within a given variety.

In general the entrance of the parasite into the watermelon roots, in the field, appears to have taken place in a manner similar to that described for cotton and alfalfa by Peltier, King, and Samson (12). This investigation, however, indicates that direct penetration by the hyphae is much more common and frequent than was heretofore recognized. The production of new roots just below the soil line, so common in cases of plants attacked by Texas root rot, was not observed on the watermelon. Although fungous filaments were abundant in the vessels of badly infected roots, no severe plugging was noted. It appears probable that the most important damage caused by the invasion of *Phymatotrichum omnivorum* is the destruction of cortical cells. This may interfere more with the passage of water from

the soil to the xylem rather than the conduction through the vessels of the xylem.

There appeared to be no repelling action of the host-cell cytoplasm for the fungus. The accompanying figures (Plate II, B, C and G) show that fungus filaments grew in direct contact with the cytoplasm. This suggests that *Phymatotrichum omnivorum* encountered no growth-inhibiting substances in the cells of the watermelon root. The fungus filaments were, however, often somewhat smaller when intracellular than when intercellular. The fact that *P. omnivorum* grew luxuriantly on watermelon-root, stem, and leaf media is further evidence that the plant is not a resistant one. Also, when small living watermelon fruits were inoculated the fungus grew and persisted.

Decrease in the size of the nuclei in invaded cells (compare Plate III, C and F with E) no doubt indicates that the normal function of the cells is being affected.

Although the seedlings used in the penetration studies were grown under somewhat abnormal conditions, the method employed assures the worker that he is dealing with pure cultures.

The manner in which the large-cell type of hyphae surrounded and enveloped a root, then sent one or more hyphae into the host, may be compared to a large appressorium-like structure. The fungus is in direct contact with the outside of the root; both mechanisms appear to aid the development of pressure to initiate penetration. The described softening action of penetrating hyphae is probably enzymatic in nature.

Results presented show that penetration is rapid in young seedlings. Evidence that few young plants in the field are killed by *Phymatotrichum omnivorum* may be due, at least in part, to the fact that the parasite may not be active early in the season when the soil is cool. Of course, there are other important factors to be considered. Why cotton seedlings grown in pots of inoculated soil do not succumb sooner than they do is not well understood.

Pure-culture studies show that *Phymatotrichum omnivorum* is inhibited or killed by the presence of *Trichoderma lignorum*. More work should be done on the effect of mixed cultures on *P. omnivorum*. This appears to be a promising field for the study of the control of Texas root rot.

SUMMARY

Watermelon, *Citrullus vulgaris*, has been repeatedly found susceptible to Texas (cotton) root rot in commercial planting in southern Arizona.

The causal organism, *Phymatotrichum omnivorum*, has been isolated from the roots of the dying watermelon vines, grown in pure culture, and has proved pathogenic on watermelon and cotton under sterile conditions

in the laboratory and in the field. All 4 varieties of watermelon tested were susceptible but there is need for further work to determine if any commercial varieties are resistant.

Cytological studies on the penetration of *Phymatotrichum omnivorum* into the roots of watermelon under pure culture conditions and field conditions show that the initial invasion of the host tissues is often a wedging of the hyphae between the host cells. Penetration of the host-cell walls appears to be due to pressure plus a softening of the cell wall at the point of contact, probably by enzymatic action.

The mycelium in the host tissue is either intra- or intercellular, but the latter condition usually is evident first. The individual cells are quite variable in size and shape and are often multinucleate.

The nuclei of invaded cells are much reduced in size and more dense than those of noninvaded cells.

Pure-culture studies show that *Phymatotrichum omnivorum* is inhibited or killed by the presence of *Trichoderma lignorum*. The possible effect of mixed cultures on the persistence of *P. omnivorum* in soils should be investigated.

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FUSARIUM YELLOWS OF CELERY[1]

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INTRODUCTION

Fusarium yellows of celery[2] was first reported in 1914 by Coons (9) in the vicinity of Kalamazoo, Michigan, where it had been known to exist for 5 years before that time. It appeared in the field in small areas that became larger each succeeding year. Coons and Nelson (10) established the fact that the causal organism was a vascular Fusarium. Early attempts at control were founded upon the substitution of highly resistant varieties of the green type for those of the self-blanching type, but the limited market demand for the former required the abandonment of this method. In 1919 a breeding program was initiated at Michigan State College that resulted in the development of resistant strains of the self-blanching type (10, 20, 21). The disease has been reported in several northern States since 1914 (10). It was found in Wisconsin in 1931, when celery infected with the yellows organism was sent to the University from Green Bay. A badly diseased field was discovered near Waukesha the following year. The fact that the disease is gradually becoming more widespread indicates the possibility that it may assume serious proportions in the future in those localities where it has not yet gained a foothold.

The present paper is a report of studies of the symptoms of the disease, the pathogenicity and variability of the causal organism, the soil temperature and moisture relations of host and parasite, and the resistance of certain varieties of celery. An exhaustive taxonomic study of the parasite was not made, since this was already under way at Michigan State College. The several isolates used were compared with a culture furnished by Ray Nelson of that institution and were found to conform with the Michigan strain in their major morphological and cultural characters and in pathogenicity.

METHODS AND MATERIALS

Isolates of the celery-yellows organism were secured from 3 sources. Isolate 5 was obtained from M. E. Anderson of the Department of Plant Pathology, University of Wisconsin, who had isolated it from diseased celery plants sent to the University from Green Bay, Wisconsin, in 1931. Isolate 6 was received from Ray Nelson of Michigan State College, who

[1] The writer expresses his sincere appreciation to Dr. J. C. Walker for the valuable suggestions offered during the course of these investigations and for aid in preparation of the manuscript.

[2] This is not to be confused with the virosis of celery known as "yellows."

stated it to be one of his most virulent cultures. A number of isolations were made from diseased Golden Plume celery collected in a field at Waukesha, Wisconsin, in the summer of 1932. Isolates 14, 16, and 18 from this source were selected for study.

Varieties of the self-blanching type of celery on the whole are susceptible to yellows, while those of the green type are resistant. In the studies undertaken Golden Self-Blanching variety was employed as representative of the former type and Winter Queen of the latter. Seed lots of these varieties were obtained from the Olds Seed Company of Madison, Wisconsin. The following additional varieties were procured from the Grand Rapids Growers Inc., Grand Rapids, Michigan: Curly Leaf Easy Bleaching, a green type recommended for badly diseased fields; M. S. C. strain of dwarf Golden Self-Blanching, a yellows-resistant strain developed at Michigan State College, which, for the sake of brevity, will be referred to as Michigan G. S. B.; Michigan Golden, a strain of tall Golden Self-Blanching, also developed at Michigan State College and resistant to yellows. The last 2 varieties are reported to be resistant under field conditions in Michigan (21).

For the most part artificially infested soils were employed. A standardized method of inoculation was followed. One-quart Mason jars containing 300 grams of corn-meal-sand medium were autoclaved and a small quantity of spore suspension introduced. After the jars were incubated at room temperature for at least 2 weeks, their contents were incorporated in enough soil to fill a 6-inch soil-temperature-tank can, which held approximately 3,400 grams of soil. The soil employed consisted of a mixture of equal parts of sandy loam and muck. This mixture had a water-holding capacity of between 70 and 80 per cent. The naturally infested soil used was secured from a diseased field at Waukesha, Wisconsin.

Temperature and pathogenicity studies were carried out in the Wisconsin soil-temperature tanks described elsewhere (14). Plants were grown in galvanized iron cans of infested soil that were held at 18° C. or below for 5 days to allow the plants to recover from transplanting before they were placed in tanks at the desired temperature. In recording data, calculations were made from the time of planting rather than from the time the cans were placed in the tanks. The common air temperatures varied from 16° to 20° C. Soil moisture was held at 70 per cent of the water-holding capacity, unless otherwise designated.

Seedlings were grown in flats of yellows-free soil and transplanted when they had reached a height of about 4 inches. Plants were not recorded as diseased until the chlorotic symptoms appeared, since stunting, although often the first symptom was also caused by unfavorable growing conditions and might, therefore, be misleading.

SYMPTOMS OF FUSARIUM YELLOWS

The symptoms of celery yellows have been described by Coons (9) and Thomas (26); but, for the sake of clarity, they will again be reviewed in the light of the 2 seasons' observation on the part of the writer in a diseased field at Waukesha, Wisconsin, and in experimental trials in the greenhouse.

Stunting of the plants is the first visible symptom, which is quickly followed by a general chlorosis that begins with the larger veins of the leaves and progresses outward. This chlorosis is quite distinctive and usually is readily distinguished from chlorosis due to other causes. Often a slight downward curling of the leaflets precedes the chlorotic stage. The type of chlorosis induced under greenhouse conditions is similar to that induced in the field. Minor differences associated with different strains of the parasite will be discussed later in this paper. Occasionally, only a part of the plant shows chlorosis but, in such a case, all affected leaves are confined to the same side of the plant. Unilateral chlorosis of leaves sometimes occurs.

Concurrently with the chlorosis of the foliage, many of the roots reveal a general brownish discoloration and decay at the tips. The vascular tissue of the primary root usually shows a reddish brown discoloration, which extends into the stem and petioles as the disease progresses. Under field conditions the crown often splits and decays. This is probably due to secondary infection, for, under greenhouse conditions, it is not observed commonly. The plants gradually die with little wilting, except at high air temperatures. When older plants are affected, the symptoms appear first in the younger leaves. Such plants may remain alive throughout the season but their growth is decidedly stunted. Severity of the disease in the field is largely conditioned by the size of the plants and by the environmental conditions.

EXPERIMENTAL RESULTS

Pathogenicity of the Isolates

In testing the pathogenicity of the several cultures obtained, the gross cultures as isolated were used rather than monoconidial lines derived from them, for it was thought that by using the latter some potentialities of the fungus might be lost. Following the standardized procedure described, the pathogenicity of isolates 5, 6, 14, 16, and 18 was determined. Soils infested with the several cultures were planted with Golden Self-Blanching and Winter Queen celery and held at a temperature of 24° C. All isolates were pathogenic on Golden Self-Blanching, while Winter Queen was resistant in all instances. However, the degree of virulence and the symptom expression varied somewhat with the isolate. To establish further proof that the designated fungus was the causal agent, sections adjacent to pieces of roots

and stems that yielded the fungus upon plating were fixed and examined. The vessels showed the presence of numerous fungal strands. The reisolates were also pathogenic. The above studies indicated that the disease, under greenhouse conditions, manifests itself in a definite way that is not unlike its appearance under field conditions; namely, by the successive appearance of a slight downward curl of leaflets, by chlorosis, and death. Distinct wilting is uncommon.

The chlorosis was of 2 types, depending upon the cultures. Isolate 5 induced a general chlorosis of the entire foliage, which, on close examination, appeared as a yellowed flecking due to a clearing of the tissue in the vicinity of the veins and fine veinlets and to the fact that the small islands of leaf tissue subtended by the veinlets were in a state of progressive chloro-

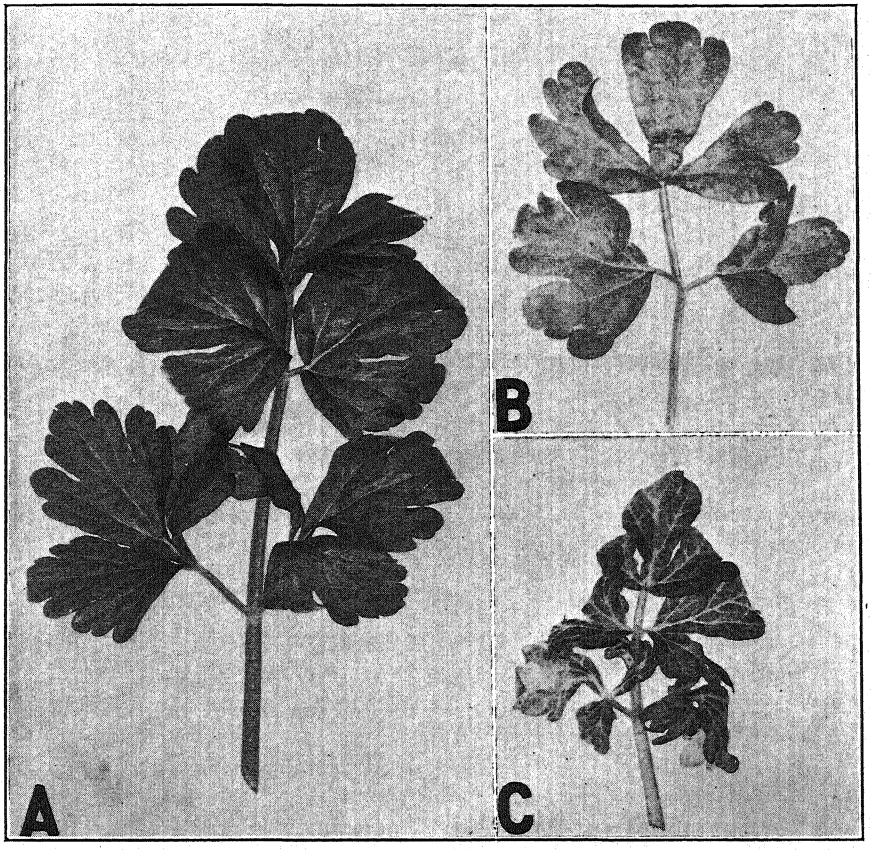


FIG. 1. Comparison of the 2 types of chlorosis produced on Golden Self-Blanching celery by different isolates. A. Leaf from a healthy plant. B. Leaf showing the general chlorosis induced by isolate 5. C. The type of chlorosis produced by the other isolates. In this type the initial chlorosis is confined to the larger veins.

TABLE 1.—*Development of yellows in Golden Self-Blanching and Michigan G. S. B. celery in soil infested with several isolates and in naturally infested soil held at temperatures of 24° and 28° C.*[a]

Soil temperature	Days after planting	Diseased plants in										
		Soil inoculated with										Naturally infested soil
		Isolate 5		Isolate 6		Isolate 14		Isolate 16		Isolate 18		
		Golden Self-Blanching	Mich. G. S. B.	Golden Self-Blanching	Mich. G. S. B.	Golden Self-Blanching	Mich. G. S. B.	Golden Self-Blanching	Mich. G. S. B.	Golden Self-Blanching	Mich. G. S. B.	Golden self-Blanching
° C.		*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
24	22	0	0	55	17	75	0	33	0	25	17	0
	27	50	0	64	17	83	17	83	17	83	17	0
	32	92	0	100	33	83	50	83	67	92	17	0
	37	92	0	100	83	83	67	83	83	92	33	0
	43	92	0	100	83	83	67	83	83	92	33	17
28	22	0	0	90	0	83	100	75	67	67	0	0
	27	80	83	90	83	83	100	83	100	83	83	17
	32	90	100	90	83	83	100	100	100	92	100	33
	37	90	100	90	83	92	100	100	100	92	100	50
	43	100	100	100	100	92	100	100	100	100	100	83

[a] There were from 10 to 12 plants of Golden Self-Blanching and 6 of Michigan G. S. B. for each lot of soil at each temperature. The results from lants in noninfested soil are omitted, since they all remained healthy.

sis. This was in contrast to the chlorosis produced by the other isolates in which there was at first a clearing only in the region of the larger veins, while the remainder of the leaf tissue was green or only slightly yellowed. Eventually all tissue became chlorotic, although it was observed in several instances that plants died before this took place. The 2 types of chlorosis are illustrated in figure 1.

In the second experiment the relative virulence of the isolates upon Golden Self-Blanching and Michigan G. S. B. at soil temperatures of 24° and 28° C. was studied. Equal parts of soil were inoculated with each culture and planted to the 2 varieties. In addition, cans of naturally infested soil that had been diluted by one-half with a sandy loam were planted with Golden Self-Blanching. Plants of both varieties in noninfested soil served as controls (Table 1). The difference between the isolates in symptom expression was similar to that noted in the previous experiment. In this trial, isolate 5 was lower in virulence than isolate 6 or 14. It apparently takes isolate 5 longer to become established in the soil, since, in later trials with soil that had produced several crops of diseased plants after the original inoculation, this strain was as virulent as isolate 14 and more virulent than isolate 6.

The 2 varieties of celery reacted generally in the same manner under the conditions of the experiment. Disease inception at 24° C. was slightly slower in Michigan G. S. B. than in Golden Self-Blanching. The incubation period for Golden Self-Blanching in newly infested soil held at 24° was about 20 days. In other trials it was found that within certain limits the longer the soil had been infested the shorter was the incubation period. Disease development was more rapid in the artificially infested soils used than in the naturally infested one. This probably can be accounted for by the fact that a greater quantity of inoculum was present in the artificially infested soil.

Environmental Relations

The relation of environment to disease is best approached, insofar as is possible, through the study of a single environmental factor upon the parasite and the host separately and upon the interrelation of both in disease development. This method was used, particularly with temperature, in the study of celery yellows. The effect of temperature upon the growth of the causal organism was first considered. This was followed by a study of the effect of both soil temperature and soil moisture upon host development. The relation of these factors to the development of yellows was then investigated.

Relation of Temperature to Fungus Growth

Isolates 5, 6, 14, and 18, together with 4 monoconidial lines from isolate 14 and 2 monoconidial lines from isolate 16, were first grown on potato-

dextrose-agar plates. They were then transferred to fresh agar plates to be used for the temperature studies. Each culture of the fungus was thus prepared in triplicate for each of the following temperatures: 8°, 12°, 16°, 20°, 24°, 28°, 32°, and 36° C. The diameter of each resulting colony was measured daily. All isolates reacted essentially alike. The absolute minimum and maximum temperatures were not determined, but the cultures showed slight growth at 8° and 36° C., good growth from 20° to 32° C., while the optimum fell close to 28° C. The data from isolate 14 is illustrated in graphic form in figure 2. The growth of isolate 5, 7 days after inoculation, is illustrated in figure 3. The average diameter of the colonies of any culture at all temperatures up to 32° C., when plotted against time, gave a straight-line curve, indicating that there was very little staling up to the time that the fungus had covered the surface of the Petri dish.

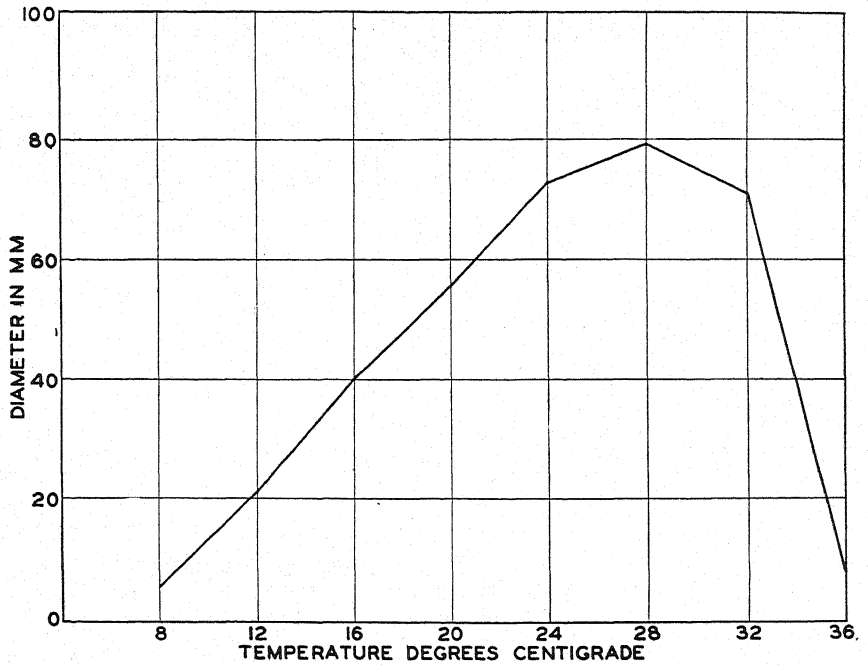


FIG. 2. Graph showing the average diameter of colonies of isolate 14 on potato-dextrose agar at various constant temperatures 6 days after inoculation.

Relation of Soil Temperature and Moisture to the Celery Plant

Six-inch cans of noninfested soil, each containing six plants of Golden Self-Blanching celery were subjected to various degrees of moisture and soil temperature. One can of soil, held at 50 per cent of its water-holding capacity and one at 70 per cent, were held at each of the following soil tem-

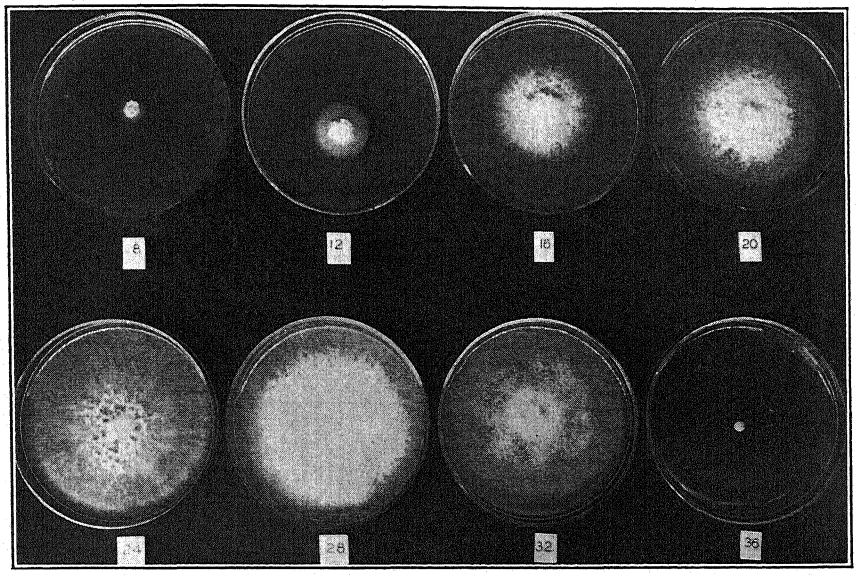


FIG. 3. Relation of temperature to the growth of isolate 5 on potato-dextrose agar. Representative colony at each of the designated temperature (°C.) 7 days after inoculation.

peratures: 18°, 20°, 22°, 24°, 26°, 28°, 30°, and 32° C. After 6 weeks the plants were removed and the soil washed from the roots. Measurements and dry weights are given in table 2. There was little difference in the growth of tops and roots at temperatures ranging from 18° to 28°, but at 30° and 32° distinct retardation occurred. This was shown not only by reduction in top height and the dry weights of tops and roots, but by chlorosis of the foliage and brown discoloration of the roots. When the plants at the 2 soil moistures are compared, it is shown that the best growth at any given temperature occurred in the higher soil moisture. The effect of low soil moisture was most severe at 30° and 32° C. Even higher moisture than 70 per cent of the water-holding capacity is tolerated by celery, since in other experiments where an occasional can leaked the plants grew well in nearly saturated soil.

Relation of Soil Temperature and Moisture to Development of Yellows

Preliminary studies on the effect of soil temperature showed that the critical low temperature for disease manifestation was somewhat below 20° C., while the optimum was around 28°. Further experiments will be reported in detail.

TABLE 2.—*Effect of soil temperature and moisture on the growth of Golden Self-Blanching celery*

Soil temperature	At 50 per cent of the water-holding capacity of the soil					At 70 per cent of the water-holding capacity of the soil				
	Plants	Tops		Average dry weight of roots	Ratio of dry weight of roots to dry weight of tops	Plants	Tops		Average dry weight of roots	Ratio of dry weight of roots to dry weight of tops
		Average height	Average dry weight				Average height	Average dry weight		
° C.	*Number*	*Cm.*	*Gm.*	*Gm.*		*Number*	*Cm.*	*Gm.*	*Gm.*	
18	5	15.2	.48	.15	1: 3.2	6	16.3	.92	.20	1: 4.5
20	6	12.8	.28	.08	1: 3.5	6	18.8	1.16	.19	1: 6.1
22	6	17.5	.68	.14	1: 4.9	6	18.7	.92	.15	1: 6.1
24	6	17.2	.66	.16	1: 4.1	6	20.2	1.19	.17	1: 7.0
26	6	15.7	.47	.09	1: 5.2	6	18.3	.80	.14	1: 5.7
28	6	16.7	.70	.12	1: 5.8	6	19.2	.99	.14	1: 7.0
30	6	9.8	.28	.05	1: 5.6	6	16.2	.70	.10	1: 7.0
32	4	8.0	.10	.03	1: 5.0	6	15.2	.66	.09	1: 7.3

In experiment 1 soil that had been inoculated some time previously with isolate 5 was placed in soil cans and planted with Golden Self-Blanching and Michigan G. S. B. celery. Three cans of infested soil for each variety were placed in soil-temperature tanks at each of the following temperatures: 18°, 20°, 24°, 26°, 28°, and 30° C. There were 7 plants to each can and the soil moisture was held at 65 per cent of the water-holding capacity. The degree of chlorosis, whether slight or marked, was recorded up to the time that plants began to die, after which time exact counts of diseased and dead plants were made (Table 3).

Yellows developed in Golden Self-Blanching at all temperatures. It appeared first at 28° and 30° where symptoms were markedly advanced at 15 days. It was progressively slower in appearance and rate of advance as the soil temperature lowered. At 18° it was not recorded until the 26th day, but by the 35th day 76 per cent of the plants were dead at this temperature. In later experiments, in which freshly inoculated soil was used, the incubation period was longer but the relative effects of the various temperatures were the same.

In the resistant variety, Michigan G. S. B., the effect of soil temperature was of the same order. This variety, however, exhibited distinct resistance at 18°, where no symptoms appeared. At progressively higher temperatures resistance decreased gradually until at 30° there was essentially no difference between the two except for the slightly slower rate of disease development in Michigan G. S. B.

In experiment 2 the soil used in the previous experiment was replanted with Golden Self-Blanching and Curly Leaf Easy-Bleaching celery and held at temperatures of 24°, 26°, 28°, 30°, 31°, and 32° C. The disease development in Golden Self-Blanching at 24°, 26°, and 28°, was similar to that in the preceding experiment except that symptoms appeared several days sooner. The disease was slightly more severe at 30° and 31° than at 28°, but development was slightly slower at 32°. At 30°, 31°, and 32° the control plants were stunted and somewhat chlorotic but the chlorosis was different in appearance from that induced by the fungus. Curly Leaf Easy-Bleaching plants remained healthy at 24° and 26° C. At 28° C. all plants were stunted and some showed typical disease symptoms, while at 30°, 31°, and 32° yellows was still more marked.

In experiment 3 the effect of soil temperature and soil moisture on the Golden Self-Blanching was studied, with 3 lots of soil infested with isolates 5, 6, and 14, respectively. One can of soil for each isolate at 50 per cent and one at 70 per cent of the water-holding capacity of the soil were used at the following soil temperatures: 18°, 22°, 24°, 26°, 28°, 30°, and 32° C. The results (Table 4) in the more moist soil were essentially like those cited in experiment 1 except that the disease was slower in appearing at 18° C.

TABLE 3.—*The effect of soil temperature upon development of yellows in Golden Self-Blanching and in Michigan G. S. B. celery*

Variety	Temperature	Total plants	After 15 days		After 20 days		After 26 days		After 35 days	
			Diseased	Dead	Diseased	Dead	Diseased	Dead	Diseased	Dead
	° C.	*Number*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Golden Self-Blanching	18	21	0	0	0	0	slight	0	91	76
	20	21	0	0	0	0	86	0	95	95
	24	21	slight	0	95	0	95	95	100	95
	26	21	do	0	100	91	100	95	100	95
	28	21	marked	0	100	86	100	95	100	95
	30	21	do	0	95	95	100	95	100	95
Michigan G. S. B.	18	21	0	0	0	0	0	0	0	0
	20	21	0	0	0	0	slight	0	slight	0
	24	21	slight	0	29	0	81	29	81	33
	26	21	do	0	14	0	100	29	100	86
	28	21	do	0	100	10	100	59	100	76
	30	21	do	0	100	24	100	86	100	91

(Table 4, Fig. 4). Isolate 6 was somewhat less virulent than the other 2 cultures. This difference in soil held at 20° C. is illustrated in figure 5, A. The vein chlorosis associated with isolates 6 and 14 and the general chlorosis associated with isolate 5 were consistent at all temperatures except 30° and 32° where chlorosis tended to become more diffuse with the first 2 strains.

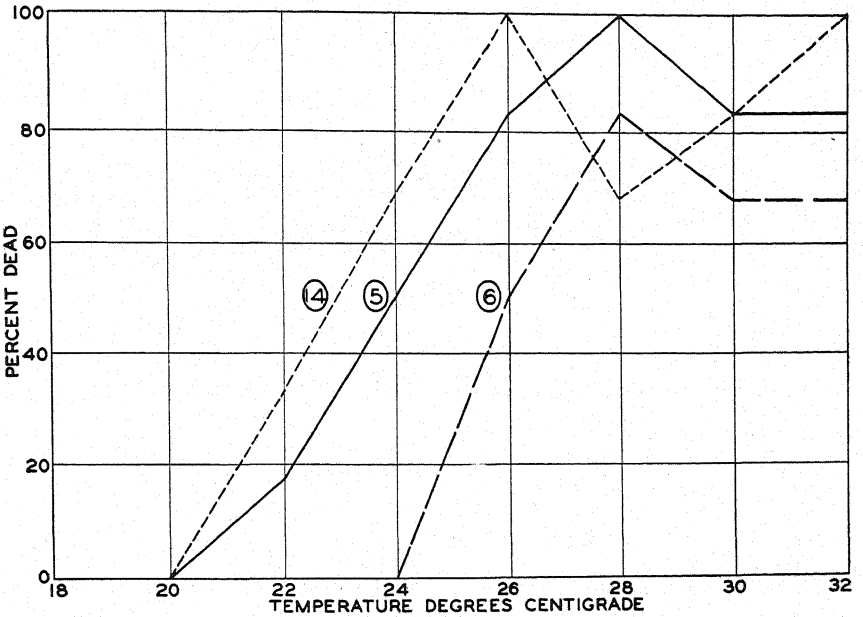


FIG. 4. Graph showing the percentage of Golden Self-Blanching plants killed with yellows after 28 days in soils infested with isolates 5, 6, and 14, respectively, and held at various constant temperatures and at 70 per cent of the water-holding capacity (Table 3).

With each isolate, disease development was decidedly slower in the dry than in the moist soil (Fig. 5, B). As noted above, the celery plant grows more slowly in the drier soil, and in plants thus stunted the disease always appeared more tardily than in vigorously growing individuals. This observation is similar to that of tomato wilt, in which Clayton (7) noted slower disease development in plants retarded by low soil moisture. The vein chlorosis in plants infected by isolates 6 and 14 was less distinct at low soil moisture.

The temperature range of the celery yellows organism is quite similar to the ranges of the other vascular Fusaria. Its growth curve follows closely that of disease development up to the optimum at about 28°, but above that temperature disease incidence does not drop off so abruptly as does radial expansion of the fungus. The disease develops at as high temperatures as

are favorable for the growth of the host. The minimum constant soil temperature for the disease was not determined, but at 18° it develops quite slowly. These results are in accord with the field observations of Nelson and Cochran (21), who reported that in the relatively warm summers of 1929, 1930, and 1931 susceptible varieties in Michigan were severely attacked, while during cooler summers the disease was of less importance.

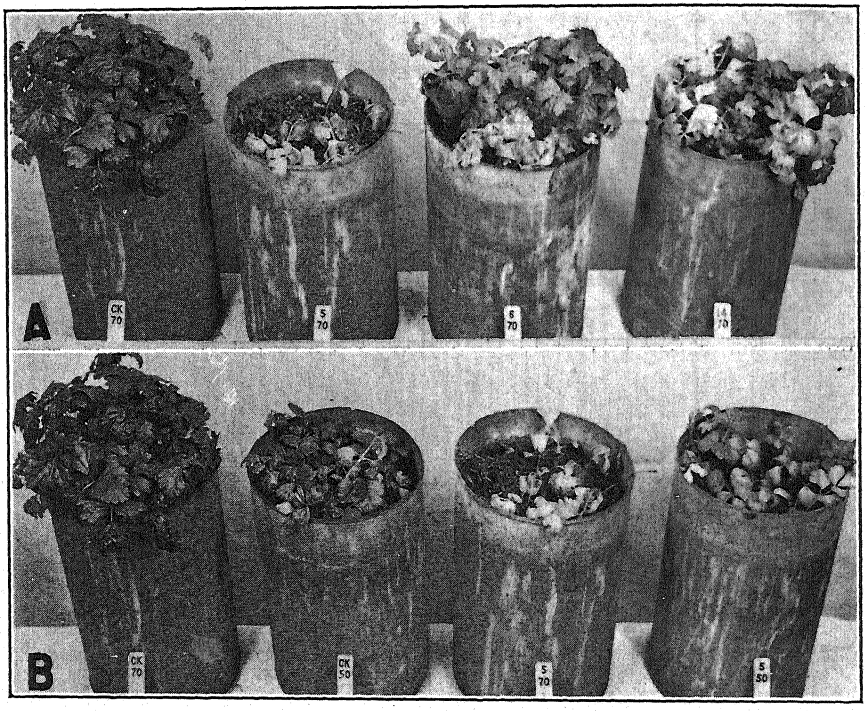


FIG. 5. Golden Self-Blanching celery 1 month after transplanting. A. Left to right, noninfested soil, soil infested with isolates 5, 6, and 14, respectively. Note greater virulence of isolate 5. Soil held at 20° C. and 70 per cent of the water-holding capacity. B. Left to right, noninfested soil at 70 per cent water-holding capacity; same at 50 per cent water-holding capacity; soil infested with isolate 5 at 70 per cent water-holding capacity; same at 50 per cent water-holding capacity. All at soil temperature of 20° C. Note greater growth of the host and greater severity of the disease at the higher soil moisture.

VARIETAL RESISTANCE

Nelson and Cochran (21) list the celery varieties grown in Michigan in 4 groups, according to their susceptibility to yellows, as follows: *Very Susceptible*:—Golden Phenomenal, Hoover Special, tall strain of Golden Self-Blanching, and dwarf strain of Golden Self-Blanching; *Susceptible*:—Florida Golden and Golden Prize; *Moderately Resistant*:—Easy Blanching

TABLE 4.—*Development of yellows in Golden Self-Blanching celery in soil inoculated with isolates 5, 6, and 14 and held at various soil temperatures and moistures*

Soil temperature	Plants dead after 28 days[a]					
	Isolate 5		Isolate 6		Isolate 14	
	50 per cent w. h. c.[c]	70 per cent w. h. c.	50 per cent w. h. c.	70 per cent w. h. c.	50 per cent w. h. c.	70 per cent w. h. c.
° C.	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
18	0	0	0	0	0	0
20	0	0	0	0	0	0
22	0	17	0	0	0	33
24	0	50	0	0	0	67
26	83	83	0	50	33	100
28	100	100	17	83	83	67
30	83	83	20[b]	67	80[b]	83
32	83	83	0	67	0	100

[a] 6 plants in each case except where indicated otherwise.

[b] 5 plants used.

[c] w. h. c. = water-holding capacity of the soil.

(Newark Market), Early Fortune, Golden Plume (Wonderful) and Golden Detroit; *Highly Resistant*:—Michigan Golden, and M. S. C. strain of dwarf Golden Self-Blanching. In the experiments already described it was shown that, as the soil temperature increased, resistance in Michigan G. S. B. and in Curly Leaf Easy-Bleaching became less pronounced. Golden Self-Blanching, was susceptible from 18° to 32°, although occasional plants remained healthy. Michigan G. S. B. was completely resistant at 18° but, as the temperature increased, typical yellows developed, although more slowly than in susceptible Golden Self-Blanching. Curly Leaf Easy-Bleaching remained free from symptoms up to 26° C. but developed typical yellows at 28° and above. Additional experiments confirmed these results. Winter Queen also was tested and reacted similarly to Curly Leaf Easy-Bleaching. Michigan Golden was grown in infested soil repeatedly at soil temperatures up to 31° and in no case was there any development of disease symptoms. This variety, therefore, appears to be distinctive in being completely resistant, even at high soil temperatures.

The above varieties were grown under various soil temperatures and platings were made from various plant parts to determine the relation of the fungus to the host. Golden Self-Blanching, at the time that symptoms

first appeared, showed the fungus to be most abundant in secondary roots, especially in those that were discolored. It was also abundant in the primary root, but became progressively less so as the stem region was approached, and it was found only rarely in the petioles. Michigan G. S. B. was similar to Golden Self-Blanching except that the fungus was rarely found in the primary root of plants grown at 18° and 20°. Curly Leaf Easy-Bleaching and Winter Queen showed the fungus abundant in secondary roots but only rarely in the primary root, at those temperatures below 28° where symptoms did not appear. However, in diseased plants grown at higher temperatures, the fungus was abundant in the primary roots. Michigan Golden, on the other hand, never yielded the fungus from the primary root but it was recovered from the secondary roots.

Histological examinations verified the above results. Golden Self-Blanching, Winter Queen and Michigan Golden were grown in infested soils held at temperatures of 24°, 26° and 28° C. Plants were removed 30 days after planting and portions were fixed, embedded, and stained. At this time Golden Self-Blanching plants were diseased at all temperatures, Winter Queen only at 28° C. and Michigan Golden at none of these temperatures. In Golden Self-Blanching the fungus was abundant in the cortical region of the secondary roots and in the vessels of both secondary and primary roots. Instances were rare of the passage of the fungus from the cortical tissue into the stelar portions. Apparently, the inner cortical cells of older roots act as a barrier even in a susceptible variety. Within the stele the fungus was confined principally to the xylem vessels where it occurred in some instances in sufficient quantity to cause complete plugging. Occasionally, in secondary roots, the tissue adjacent to the vessels had collapsed and had been penetrated by the fungus. This condition was rare in the primary roots. With the extension of the vessels into the stem, fewer hyphae were noted and in most instances they did not occur beyond the transition region. They were only rarely observed in vascular tissue of the petioles.

Winter Queen, at temperatures that did not induce disease symptoms, showed the fungus abundant in the cortex and rare in the xylem of secondary roots. Diseased plants of this variety showed the fungus as widely distributed as in Golden Self-Blanching. In Michigan Golden the fungus was present in the cortex of secondary roots but never in the stele. The relation of the fungus to the cortical cells of all 3 varieties was essentially the same, the fungus being either inter- or intracellular.

The above results may be compared with those secured by others in varieties of cabbage resistant to *Fusarium conglutinans* Wr. Anderson (1) found in the Wisconsin Hollander cabbage that, with the rise in soil temperature, increasing percentages of diseased plants appeared until, at

24° C., resistance usually disappeared entirely. This variety is somewhat analogous to Michigan G. S. B. celery in that resistance is confined to relatively low soil temperatures. Walker and Smith (28) showed that in homozygous resistant lines of cabbage at soil temperatures of 26° and above, symptoms were produced but they were atypical and the fungus was not isolated from the tissue. The situation is somewhat different in celery yellows, in that those varieties that become diseased only at soil temperatures of 28° C. and above, such as Winter Queen and Curly Leaf Easy-Bleaching, develop typical symptoms and the fungus is present in the host tissue. Michigan Golden is most comparable to homozygous resistant cabbage, since it is completely resistant at all temperatures and fungus invasion is confined to the outer cortical cells of secondary roots.

VARIABILITY OF THE PARASITE

It has been shown already that the several isolates varied in their degree of virulence and in the type of symptom produced upon the host. Further studies were made with isolates 5, 14, and 16, which at the time had been carried in pure culture for 2 years or more. The range of variation in cultural characters was investigated first, followed by studies in pathogenicity. Monoconidial lines were isolated following the method described by Keitt (16). Microconidia were used since cytological studies indicated that they were all uninucleate. Cultural characters were studied on potato-dextrose agar that had been thoroughly mixed before pouring. A single lot of medium was used for each series.

In the first series of monoconidial cultures a wide range of variability was noted in those derived from isolates 5 and 16, while those from isolate 14 were very similar to one another. The 31 cultures derived from isolate 5 were placed in 5 groups and the 22 lines from isolate 16 were placed in 3 groups. Thus 9 groups were distinguished from one another on the basis of the kind and amount of aerial mycelium, the type of subaerial mycelium, and the degree of pigmentation of the substrate. Two cultures were selected from each distinct group and 10 conidia were isolated from each. This procedure was continued for 4 conidial generations. Thus about 750 microconidia were isolated and cultured and their thalli studied. There was a tendency toward stability in cultural characters, although some segregation continued to occur in some of the lines.

Nine monoconidial lines that showed the highest degree of constancy were then selected as representative of the various culture types. Ten monoconidial subcultures were made from each and this procedure was repeated through 10 successive conidial generations. Eight of the lines remained constant in culture type. One line segregated in the fifth conidial generation. The divergent form remained constant through the five suc-

ceeding generations. The growth rate of all these cultures on potato-dextrose agar was essentially the same at 24° C.

The 9 original cultures just mentioned were tested for pathogenicity at once and again after they had remained in culture tubes for about 2 months. The most susceptible variety, Golden Self-Blanching, and the most resistant variety, Michigan Golden, were included in the pathogenicity tests. The latter remained free from symptoms in all cases, while the disease developed as usual on the former. All of the cultures derived from isolate 5 produced the general chlorosis typical of that isolate, while those derived from the other isolates produced symptoms characteristic of their respective sources. It was evident that this property was very well fixed in the respective isolates. The 9 original lines varied from high to low virulence. This was consistent in each of 3 pathogenicity trials. The relative virulence of the 9 original cultures is illustrated in figures 6 and 7.

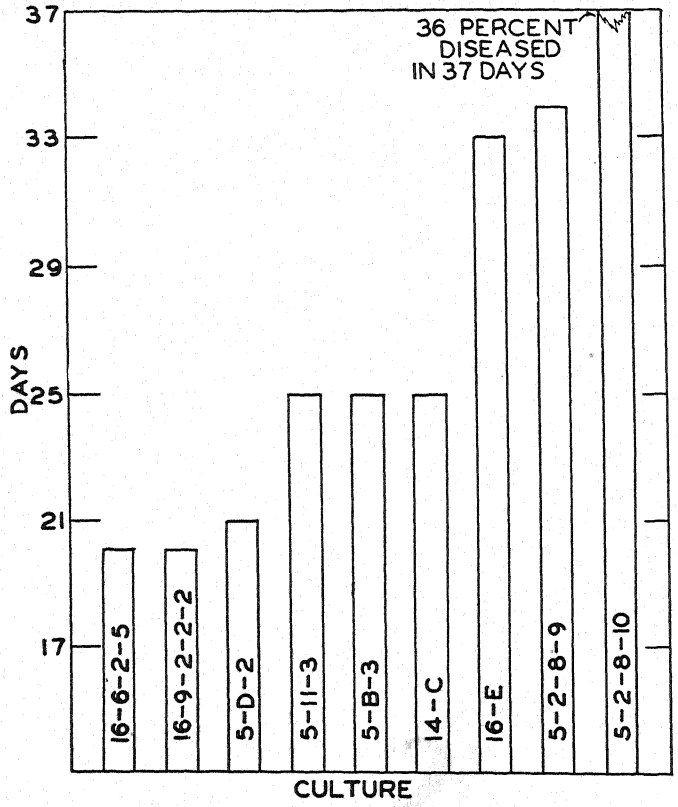


FIG. 6. Comparison of the number of days required for each of 9 monoconidial strains to produce the disease in 75 per cent of plants. 28 plants were used for each culture.

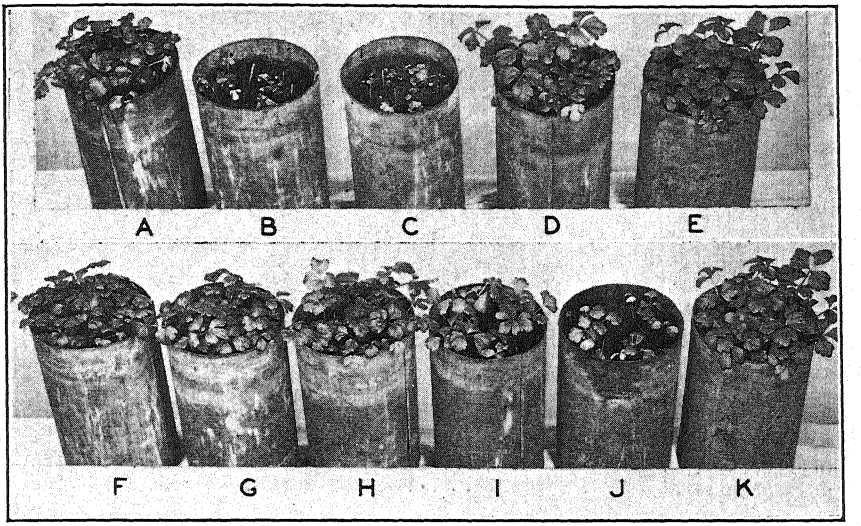


FIG. 7. Comparison of the relative virulence of 9 monoconidial lines derived from 3 original isolates of the celery-yellows organism on Golden Self-Blanching celery, 39 days after planting in soil at 25° C. A was derived from isolate 14; B, C, D were derived from isolate 16; F, G, H, I, J were derived from isolate 5; E and K are controls in noninfested soil.

Reisolations were made from plants infected with each strain. In all cases but one the reisolate was similar in cultural characters to the original. In the exceptional case one reisolate produced pigment in the substrate while the other, like the original, did not. Three reisolates from low-virulent strains and 2 from high-virulent strains were subcultured. Seventy-four subcultures, thus secured, were tested for pathogenicity on Golden Self-Blanching. These showed a wide range in degree of virulence and there was no correlation with the virulence of the mother culture nor with the type of cultural character.

It may thus be concluded that there is a tendency for the monoconidial lines to approach a certain degree of constancy in cultural characters through repeated single-spore selection. This is not true with regard to degree of virulence on Golden Self-Blanching variety. Two pathogenic characters, however, did not vary. The type of symptom on the susceptible variety remained constant and the complete lack of ability of all the isolates to produce symptoms on Michigan Golden appeared to be fixed.

DISCUSSION

Soil temperature has been shown to be an important influential factor in a number of diseases caused by vascular Fusaria (2, 8, 15, 19, 27). It

has been shown in this study that the disease developed very slowly at 18° C., and that the rate increased with temperature up to 28° C. Thus the temperature curve for growth of the organism followed closely that for disease development. The influence of temperature upon the disease, therefore, appeared to result from its effect upon the parasite rather than upon the host. Soil moisture, on the other hand, affected disease development through its influence upon the host. At low soil moisture, where the growth of the celery plant was retarded, disease development was decidedly slower than at high soil moisture, which favored the host.

Soil temperature also affected the expression of resistance in certain varieties. Michigan G. S. B. was highly resistant only at 18° C., while Winter Queen and Curly Leaf Easy-Bleaching were resistant up to 26°. This indicates that the resistant principle does not function at certain soil temperatures and that the temperature range for resistance varies with the variety. Michigan Golden is outstanding in that it remained completely resistant at all temperatures to which it was exposed.

That variability occurs in the celery yellows parasite is not surprising, for it has been shown to be characteristic of a number of vascular Fusaria (5, 22, 23, 29). The variability in this group has been recognized by differences in cultural characters and in degree of virulence. It has been shown in these studies that the extent of variability in a very limited number of isolates was quite great and extended into successive generations of monoconidial cultures derived from the isolates. These differences were expressed in cultural characters, in degree of virulence, and in the type of symptom produced. It was further shown that after continued subculturing certain lines showed a marked degree of uniformity in cultural characters and that, once purified, passage through the host produced little change in most of them. This did not preclude the possibility of changes in virulence. The study of the pathogenicity of a large number of monoconidial cultures from reisolates of low- and high-virulent strains indicated that the subcultures varied considerably in virulence. The parent reisolates maintained their relative standings in this regard, although there were indications that as a result of their passage through the host they had become somewhat more virulent, particularly so in the case of the reisolates of strains of low pathogenicity. If the cultures be inconstant in virulence, then it would be expected that the reisolate would tend to show the upper virulence range of this fluctuation. On the other hand, if the same strains be grown on artificial media, the chances of perpetuating either low or highly virulent forms are equal. The apparently constant fluctuation between higher and lower virulence would suggest that it would be but a step from a low virulent form to an entirely saprophytic one, or, conversely, from a nonparasitic to a parasitic state. This might indicate that the

vascular Fusaria arose as one or more groups and that, through the fluctuation of certain of its members, forms developed that were parasitic to a specific host.

The results of these studies would further suggest that any taxonomic study of the parasite must take into consideration not only the variability in a single isolate but that of a great many taken from a wide geographical range. No evidence of selective host pathogenicity was evinced in these studies, but this cannot be regarded as conclusive of its absence until more extensive studies have been made, for, since the parasite can be sufficiently divergent to produce different symptom expressions on the same host plant, it might well extend to a differential in host specialization between strains.

The reaction of these variants raises the question of the mechanism involved. Theories have been proposed to explain variability but little real evidence has been advanced as to its cause. Leonian (17, 18) disposed of the necessity of explaining the mechanism by considering dissociation a phenomenon whereby the variability of the culture traces the limits of the species and is thus a normal behavior. This may or may not hold true of the parasite with which this paper deals.

Brierley (3, 4) considered that dissociation could be accounted for by a heterocaryotic condition of the original isolate, which he believed to be followed by a reassortment of nuclei in the variants. More recently, Hansen and Smith (12) have presented experimental data in support of this theory They believed that hyphal fusions brought about the heterocaryotic condition. Leonian (18), however, was not able to induce "Mixochimaera" in *Fusarium moniliforme* Sheldon by mixing different cultures. The variability of celery Fusarium was not of this order, for cytological study indicated that the microconidia were uninucleate. This was supported by evidence not incorporated in the above studies in which attempts were made to hybridize 2 lines of fixed culture types by mixing spore suspensions and growing the resultant cultures on plates and tube slants. Only the 2 parent types could be reisolated.

Several workers are of the opinion that certain variations shown are due to mutation (24, 25). However, the extent to which variation in this fungus occurs would necessitate the assumption that chromosome aberrations occur frequently. Caldis and Coons (6) found that more or less permanent changes could be induced by certain changes in environmental conditions, but these were negated by passing the organism back through the host. This induced variation they believed to be similar to the *Dauermodification* of Jollos (13). This would not seem to be the case with the celery Fusarium, however, for, by passing the fungus back through the host, few changes in culture type were induced. The delayed segregation of Dickinson (11) is likewise not tenable, since the sexual stage has never been

observed in any of the vascular Fusaria. Thus it appears to remain for still another explanation to account for the variability exhibited by the celery-yellows organism.

SUMMARY

This paper reports the results of studies upon the pathogenicity, the environmental relations, and the cultural and pathogenic variability of the celery-yellows organism.

The symptoms of celery yellows were quite distinct and agreed with those described by other workers. In greenhouse studies 2 types of chlorosis were distinguished, depending upon the isolate used.

Isolates obtained from 2 localities in Wisconsin agreed in pathogenicity with one obtained from Michigan but there were differences in the degree of virulence and in certain culture characters.

All isolates had essentially the same temperature relations when grown on potato-dextrose agar held at various constant temperatures. The extremes of growth were close to 8° and 36° C., good growth from 20° to 32° C. and the optimum was at about 28° C.

The celery plant is favored by soil temperature of 18° to 28° C. above which there is marked stunting.

Disease developed in the susceptible Golden Self-Bleaching variety at soil temperatures of 18° to 32° C. the latter temperature being close to the limit of host tolerance. The incubation period was shortened proportionately with the increase of temperature up to 28° C.

Soil temperature affected the resistance of certain varieties. Michigan G. S. B. was highly resistant only at 18° C., Winter Queen and Curly Leaf Easy-Bleaching up to 26°, while Michigan Golden was resistant at all temperatures studied. All varieties, whether diseased or not, showed fungus present in the cortical region of secondary roots, but only plants showing symptoms contained it in the vessels of the primary root.

At low soil moisture, where host development was retarded, the development of the disease was checked proportionately.

Monoconidial cultures of several isolates varied in cultural characters and in the degree of virulence. Continued subculturing tended to produce constancy in culture characters. On passing these through the host the cultures, for the most part, remained unchanged.

Virulence was not fixed by continued subculturing.

The type of symptoms produced remained constant in the various isolates, and all strains of the fungus failed to produce the disease in Michigan Golden variety of celery.

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QUANTITATIVE STUDIES ON THE FILTRATION OF TOBACCO-MOSAIC VIRUS[1, 2]

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INTRODUCTION

Previous investigations on the filterability of plant viruses have been either qualitative in nature or the quantity of virus in the filtrate was determined from the highest dilution that remained infectious. With the bean local-lesion method of determining the concentration of samples of tobacco-mosaic virus, developed by Price (24), dilution to the end point of infectivity is not necessary for measuring the amount of virus present in a given sample, since the number of lesions produced on the bean leaves depends on virus concentration. The method, therefore, allows a more rapid as well as more accurate quantitative study of the filterability of tobacco-mosaic virus than has been possible previously. The purpose of the studies reported in this paper was to determine the amount of tobacco-mosaic virus that passes filters of different kinds under various conditions and to estimate by ultrafiltration the size of virus particles.

REVIEW OF LITERATURE

In 1892 Iwanowski (16) obtained typical mosaic symptoms in tobacco plants inoculated with infectious juice that had passed bacteria-proof filters. He believed that a toxin secreted by a causal bacterium in diseased plants passed into his filtrate and produced the symptoms of disease. Beijerinck (5), in 1898, also obtained typical mosaic symptoms in healthy plants inoculated with a bacteria-free filtrate (Chamberland filter) of infectious juice. By successive subinoculations he proved that the disease was not caused by a filterable nonmultiplying toxin, as Iwanoski believed, but by a filterable multiplying agent, which he termed *Contagium vivum fluidum*.

Some of the virus diseases of plants reported to be produced from bacteria-free filtrates are:

Tobacco mosaic—Iwanoski (16), 1892;
Cucumber mosaic—Doolittle (8), 1920;
Healthy potato—Johnson (18), 1925;
Streak of tomato—Vanterpool (32), 1926;
Aucuba mosaic—Smith (26), 1928;

[1] Presented as part of a thesis submitted to the Graduate Faculty of the University of Minnesota in partial fulfillment of the requirements for the degree of Doctor of Philosophy; granted June 18, 1934.

[2] Published at the expense of The Rockefeller Institute for Medical Research out of the regular order of date of acceptance of the manuscript.

Curly top of sugar beet—Severin and Swezy (25), 1928;
Potato mosaic—Smith (27), 1928;
Sisal disease—Staner (29), 1929;
Ring spot of potato—Smith (28), 1929;
Yellow dwarf of onion—Melhus *et al.* (22), 1929;
Glasshouse streak of tomato—Jarrett (17), 1930;
Rose wilt and dieback—Grieve (12), 1931;
Ring spot disease of tobacco—Henderson and Wingard (15), 1931;
Hyoscyamus IV—Hamilton (13), 1932;
Streak disease of maize—Storey (30), 1932.

Filterability of tobacco-mosaic virus has been verified by several investigators, but only Chapman (7) has reported quantitative studies. On the basis of dilution capacity of the filtered and nonfiltered samples, he stated that Chamberland (grade not mentioned), Berkefeld "N," Berkefeld "W," and Kitasato filters allowed 91, 63, 47, and 40 per cent, respectively, of the virus to pass.

METHODS

Samples of tobacco-mosaic virus were obtained from plants of *Nicotiana tabacum* L. var. Turkish that had been kept in a greenhouse for about a month after infection with Johnson's virus No. 1. After harvesting and macerating the plants in a food chopper, the infectious juice was strained through double thickness of cheesecloth under pressure of wringing by the hands, placed in test tubes, and frozen immediately. Subsequently, it was thawed and refrozen on 2 occasions before being stored in an ice-box. This treatment did not noticeably affect infectivity of the virus, but cleared the infectious juice of coarse plant-tissue fragments.

Filters employed were Berkefeld candles ("V," "N," and "W"), coarse, medium, and fine; Seitz E. K. filter pads; Kramer's plaster-of-Paris and magnesium oxide filters; Jena sintered-glass filters; Bechhold-Konig filters; and collodion ultrafilters. The Berkefeld candles were used repeatedly. Between filtration experiments they were cleaned by filtering 100 cc. of boiling sodium carbonate solution (2 per cent), followed with 50 cc. of potassium permanganate solution (10 per cent), then with 50 cc. of a mixture of equal parts of hydrochloric acid (5 per cent) and sodium sulphite solution (10 per cent). After passing 200 cc. of boiling distilled water through each filter, it was sterilized by autoclaving. The other filters were used only once.

Testing of filters included the determination of pore size, imperviousness to bacteria, and the sign of the electrical charge on the filtering surfaces. The size of pores in the Berkefeld, Jena sintered-glass, Bechhold-Konig filters, and collodion ultrafilters, was determined by Bechhold's (3) air-water method. Faulty or broken filters were easily detected by the size of the bubbles that arose from the candle and by the pressure required to

force air through the pores. Filters that proved to be satisfactory by this method were further tested for their imperviousness to *Bacillus prodigiosus* Flügge. This was accomplished by filtering a suspension of the organism through the filters and plating out aseptically a given volume of the filtrate. Absence of bacterial colonies in the plates after 4 days' incubation was evidence that the filters retained the bacteria.

The sign of the electrical charge on the surface of filters was determined by filtering solutions of acid and basic dyes. Filters possessing a negative charge retain basic dyes (colored cation) because of the adsorption of positive ions to the negative surface of the filter. Those possessing a positive charge retain acid dyes (colored anion) by adsorption, while those with both negatively and positively charged areas retain both dyes. It was found in these determinations that Berkefeld candles, Seitz E. K. filter pads, and collodion ultrafilters retained Victoria Blue (basic dye) between pH 1.5 and pH 8.5, and allowed Congo Red (acid dye) between pH 5.0 and pH 8.5 to pass through. Therefore, these filters are negatively charged with respect to the dyes through the pH range at which virus suspensions (pH 1.5 to 8.5) were filtered. Kramer's plaster-of-Paris filters prepared for these studies retained both dyes. The Jena sintered-glass and Bechhold-Konig filters, due to their coarseness, allowed both dyes to pass through.

Negative pressure of 700 mm. of mercury was used throughout, unless otherwise stated. It was maintained by means of a glass water pump and was measured with a mercury manometer. Filtration was not started until the negative pressure reached the desired value.

Virus concentration was determined quantitatively by the bean local-lesion method described by Price (24). By this method infectivity of the virus is measured, and the approximate amount of virus in a given sample may be determined from the number of lesions produced. Since infectivity was found in other experiments to be optimum at pH 7.0 to 8.5 in 0.1 molar phosphate buffer, all the virus suspensions, unless otherwise stated, were adjusted to pH 8.5 in 0.1 molar disodium phosphate before inoculation. Immediately after inoculation, the plants were washed with running tap water and kept in a greenhouse, under conditions favorable for growth, until local lesions became conspicuous enough (4 or 5 days) for counting. Due to the difficulty of counting lesions in excess of 300 per leaf, the samples that were known from previous experience to contain abundance of virus were diluted before being used for inoculating beans. This was justified, since the number of lesions produced on a leaf was found in other experiments to be proportional to the virus concentration when the original virus suspension was diluted 1×10^{-4} or more in 0.1 molar disodium phosphate. The values for the concentrated virus samples given in the tables, therefore, constitute the number of lesions multiplied by the dilution value

of the virus suspension. Inoculations were made by rubbing the upper surface of the primary leaves with a bandage gauze pad previously moistened with 5 cc. of the virus suspension to be tested. All the samples of virus in a given experiment were inoculated as soon as possible to 12-day-old beans of the variety Scotia.

EXPERIMENTATION

Filtration of Virus Diluted in Distilled Water

The amount of virus in water dilutions that passes through Berkefeld "W" candles was determined by filtering 100 cc. of each dilution and measuring the virus activity of the filtered and unfiltered samples. The suspensions were applied to beans without adjusting the reaction, which varied from pH 5.6 to 6.8 as determined colorimetrically. The results are given in table 1.

TABLE 1.—*Number of lesions produced by Berkefeld "W" filtrates and by non-filtered samples of tobacco-mosaic virus at various dilutions in distilled water*

Virus dilution	pH of medium	Times repeated	Number of local lesions[a]		Virus activity in the filtrate
			Unfiltered	Filtered	
					per cent
2×10^{-1}	5.6	10	1932 ± 91.05	1532 ± 80.26	79
1×10^{-1}	5.8	10	1889 ± 163.90	1368 ± 120.73	72
4×10^{-1}	6.0	10	1479 ± 89.03	1299 ± 68.79	87
$.2 \times 10^{-1}$	6.0	10	1355 ± 103.87	1174 ± 64.07	86
1×10^{-2}	6.2	10	748 ± 24.28	694 ± 44.51	92
$.2 \times 10^{-2}$	6.4	10	518 ± 37.09	515 ± 30.35	99
1×10^{-3}	6.5	10	335 ± 32.37	387 ± 43.84	115
Check			0		

[a] = Average number of lesions on 20 beans.
± = Probable error.

These measurements show that the Berkefeld "W" filtrate was at least 75 per cent as infectious as the unfiltered sample. They also show that a greater percentage of the virus filters through at high dilutions than at low dilutions. At 1×10^{-3} dilution the filtered suspension was 15 per cent more infectious than the unfiltered. This suggests that filtration may disperse the virus or remove an inhibitory substance and thus result in an increased number of active infective units. Because of the effect of filtration on virus infectivity (conclusively shown later in this paper), the quantity of virus in filtered samples as compared with the quantity in unfiltered samples is not determined accurately by the local-lesion method. The number of lesions produced from a given sample, however, depends upon the approximate amount of virus in the suspension.

Filtration of Virus at Various Hydrogen-ion Concentrations

The amount of virus passing through Berkefeld "W" candles at reactions of various pH values was determined in the following experiments. Virus (100 cc.) at a 1×10^{-1} dilution in 0.1 molar phosphate buffer (pH 8.5, 5.6, 4.5, 3.0, and 1.5) and in distilled water (pH 5.6) was filtered. The residue was resuspended in 0.1 molar disodium phosphate. Before estimating virus infectivity in the various samples by inoculating beans, the reaction was adjusted to pH 8.5 in 0.1 molar phosphate buffer. Results of these measurements are given in table 2.

TABLE 2.—*Filterability of tobacco-mosaic virus at reactions of various pH values through Berkfeld "W" candles*

Virus suspension	Unfiltered	Filtrate	Residue
Buffered at pH 8.5	85,890[a] ± 5,925.2	142,356 ± 8,183.70	2,324 ± 647.3
Without buffer pH 5.6	74,113 ± 13,131.4	32,797 ± 1,137.20	108,853 ± 2,230.5
Buffered at pH 5.6	105,300 ± 4,241.7	25,953 ± 2,687.20	92,142 ± 1,350.1
Buffered at pH 4.5	64,550 ± 646.9	30.7 ± 2.99	104,958 ± 986.4
Buffered at pH 3.0	121,500 ± 7,756.7	2.3 ± .337	126,937 ± 4,562.5
Buffered at pH 1.5	134,000 ± 6,340.3	0.0	106,032 ± 6,205.3
Check	0		

[a] = Average number of local lesions on 20 beans.
± = Probable error.

These data show that the reaction of the suspension determines the amount of virus passing through Berkefeld "W" candles. At pH 8.5 the filtered virus was 66 per cent more infectious than the unfiltered. This increase was probably due to virus dispersion and not to the removal of an inhibiting substance, since the residue left on the filter had no detectable effect upon the infectivity of added virus. The fact that the residue was almost inactive indicated that nearly all of the virus filtered through at this reaction. A part of the virus passed through in acid media of pH 5.6, 4.5, and 3.0. According to these data, the filtrates were 44.2 and 24.6 per cent as infectious as the unfiltered juice at pH 5.6 in buffered and unbuffered suspensions, respectively, while at pH 4.5 and 3.0 they were 0.047 and 0.0019 per cent as infectious. Virus was not demonstrated in the filtrates at pH 1.5. Nonfilterable virus in the residue was recovered by elution in 0.1 molar disodium phosphate.

Fractional Filtration of Virus through Berkefeld "W" Candles

The amount of virus in various portions of the filtrate was determined by fractionation experiments in which virus (100 cc.) at a 1×10^{-1} dilution in 0.1 molar phosphate buffer (pH 8.5, 5.6, 4.5, 3.0, and 1.5) and in distilled water (pH 5.6) was filtered through Berkefeld "W" candles. The filtrates were fractionated into 10 portions of 10 cc. each as the liquid passed through the candles. Results of the measured virus infectivity of the various fractions are given in table 3.

TABLE 3.—*Virus infectivity of successive 10 cc. fractions of Berkefeld "W" filtrate of tobacco-mosaic virus at reactions of various pH values*

Fraction	Average number of lesions on 20 beans					
	Without buffer	With 0.1 M phosphate buffer				
	pH 5.6	pH 8.5	pH 5.6	pH 4.5	pH 3.0	pH 1.5
1st 10 cc.	8,882 ±	142,540 ±	16,850 ±	70 ±	4 ±	0
	1,127.7	13,073.7	1,922.3	22.3	1.34	
2nd 10 cc.	31,280 ±	145,340 ±	30,900 ±	80 ±	2 ±	0
	2,918.8	15,843.7	3.844.6	13.4	1.34	
3rd 10 cc.	41,380 ±	141,740 ±	32,800 ±	60 ±	4 ±	0
	4,948.6	12,787.3	5,935.6	26.9	1.34	
4th 10 cc.	50,380 ±	138,000 ±	43,000 ±	80 ±	3 ±	0
	7,767.6	5,765.8	1,618.8	0.0	.67	
5th 10 cc.	98,960 ±	137,100 ±	84,200 ±	35 ±	4 ±	0
	6,639.8	10,713.5	2,832.9	3.3	2.69	
6th 10 cc.	23,962 ±	149,740 ±	15,980 ±	30 ±	3 ±	0
	1,997.3	7,932.5	2,778.9	6.7	.67	
7th 10 cc.	25,386 ±	148,140 ±	9,860 ±	15 ±	3 ±	0
	1,398.7	7,600.0	1,270.6	11.3	2.02	
8th 10 cc.	21,182 ±	138,540 ±	9,180 ±	0	0	0
	1,315.8	6,731.1	925.6			
9th 10 cc.	17,122 ±	146,600 ±	9,820 ±	0	0	0
	1,152.7	19,623.5	2,751.9			
10th 10 cc.	6,257 ±	165,020 ±	6,940 ±	0	0	0
	1,755.5	11,735.4	1,829.9			
Unfiltered	74,133 ±	85,890 ±	105,300 ±	64,550 ±	121,500 ±	134,000 ±
	13,131.4	5,925.2	4,241.7	646.9	7,756.7	6,340.3
Check	0	0	0	0	0	0

± = Probable error.

It is evident from these data that essentially all of the virus passed through the filters at pH 8.5, both at the beginning of filtration and thereafter. The filtered fractions were from 47 to 92 per cent more infectious than the unfiltered juice. Percentage infectivity of virus in the 10 filtered

fractions, compared with the infectivity of the nonfiltered juice, was 165.0, 169.2, 165.1, 149.0, 147.9, 174.4, 149.2, 161.2, 171.6, and 192.1, respectively.

A small amount of virus at pH 5.6 passed through the candles at the beginning of filtration. As filtration proceeded, the filtrate became more infectious until 50 cc. had filtered through when the infectivity of the 6th fraction was equal to that of nonfiltered juice. Later fractions proved to be less than 50 per cent as infectious as nonfiltered juice. Infectivity of the 10 fractions of the nonbuffered filtrates was 11.9, 42.2, 55.8, 67.9, 137.8, 32.3, 34.2, 27.2, 23.0, and 8.4 per cent of that of the nonfiltered virus, and 16.0, 29.3, 31.1, 40.7, 79.8, 15.1, 9.3, 8.7, 9.3, and 6.5 per cent of that of the buffered virus.

At pH 4.5 and 3.0, a very small amount of virus passed through the candles at the beginning of filtration and until 70 cc. had filtered, after which the filtrate was noninfectious. Infectivity of the first 7 fractions of the filtrate at pH 4.5 was 0.108, 0.124, 0.093, 0.124, 0.054, 0.046, and 0.023 per cent of that in the nonfiltered. The first 7 fractions at pH 3.0 possessed 0.0032, 0.0016, 0.0032, 0.0024, 0.0032, 0.0024, and 0.0024 per cent, respectively, the infectivity of the nonfiltered.

All fractions were noninfectious when virus was filtered at pH 1.5. The nonfiltered virus remained highly infectious at this reaction.

The data in table 3, columns 2 and 3, were subjected to the analysis of variance, as developed by Fisher (10), in order to study biometrically the differences in estimated virus concentration of the various fractions. The analysis of variance consists of sorting out experimental variability and allocating it to different sources, leaving a residual portion ascribable to uncontrolled causes designated as error. The sums of squares form the basis for the measurement of variability. The mean value of the sums of squares ascribed to any source of variation (the variance or standard deviation squared) is obtained by dividing the sum of squares by the appropriate number of degrees of freedom, where the term degrees of freedom is used in the sense of independent comparisons. The significance of the difference between the different variances is determined by the z-test. A complete discussion of the analysis of variance is given by Fisher (10) and full details as to the arithmetical procedure are discussed by Fisher and Wishart (11).

In the data from fractionation of virus filtrates at pH 5.6 without buffers (Table 3, column 2), it is necessary to know if the estimate of variance due to filtration (various fractions) is significantly greater than that due to error. From the analysis (Table 4), it appears that the data are highly significant, since the observed value of z exceeds the calculated value of z at the 1 per cent point, indicating that differences as great as or greater than those due to chance would be found less than once in 100

TABLE 4.—*Analysis of variance of data presented in table 3, column 2*

Variation due to	Sum of squares	Degrees of freedom	Variance	Z
Tests	4,345,134,198.30	4		
Fractions	40,636,824,211.25	10	4,063,682,421.1	1.4017[a]
Error	9,851,924,413.29	40	246,298,110.3	
Total	54,853,882,822.85	54		

[a] = Exceeds the 1 per cent point.

times. By applying the difference required for significance (19,852) to the means, it is evident that the first filtered fraction is significantly different from the 2nd, 3rd, 4th, or 5th fraction, and also from the nonfiltered. Moreover, the 5th fraction is significantly different from all the others.

In order to determine whether the increased infectivity of virus filtered at pH 8.5 was biometrically significant, variance of the data was analyzed. Since the observed value of z (Table 5) for the analysis of variance of

TABLE 5.—*Analysis of variance of data presented in table 3, column 3*

Variation due to	Sum of squares	Degrees of freedom	Variance	Z
Tests	26,765,504,231	4		
Fractions	19,054,757,686	10	1,905,475,768.6	0.4975[a]
Error	28,173,592,769	40	704,339,819.2	
Total	73,993,854,686	54		

[a] = Exceeds the 5 per cent point.

these results exceeds the 5 per cent point, the data are significant. By applying the required difference for significance between any two means (33,570), it is to be noted that increased infectivity in all the filtered fractions was significantly different from the control or nonfiltered.

Correlation of Clogged Pores with Virus Retention

Clogged filter pores, as evidenced by the rate of flow of liquid, were correlated with the retention of virus during the fractional filtration experiments. The time required for each 10 cc. fraction to filter through at reactions of various pH values is given in table 6.

TABLE 6.—*Rate of filtration of tobacco-mosaic virus at reactions of various pH values through Berkefeld "W" candles*

Fraction of filtrate	Time to filter 10 cc.					
	Without buffer pH 5.6	pH 8.5	pH 5.6	pH 4.5	pH 3.0	pH 1.5
1st 10 cc.	15 sec.	20 sec.	20 sec.	25 sec.	27 sec.	32 sec.
2nd 10 cc.	16 "	20 "	30 "	30 "	37 "	95 "
3rd 10 cc.	20 "	20 "	40 "	75 "	50 "	5 min.
4th 10 cc.	29 "	20 "	55 "	172 "	70 "	10 "
5th 10 cc.	49 "	20 "	135 "	4.75 min.	90 "	15 "
6th 10 cc.	1.5 min.	20 "	3.5 min.	7.25 "	115 "	20 "
7th 10 cc.	3.66 "	20 "	6.75 "	10.0 "	147 "	30 "
8th 10 cc.	6.75 "	20 "	11.0 "	12.0 "	170 "	41 "
9th 10 cc.	17.5 "	20 "	17.5 "	14.5 "	3.16 min.	60 "
10th 10 cc.	30.0 "	20 "	23.5 "	17.0 "	3.75 "	62 "

These measurements show that the pores were not clogged at pH 8.5, as was shown by the constant rate of flow during filtration. At acid reactions the pores were clogged. With the 5th fraction at pH 5.6, they were not clogged to the extent of holding back virus, while afterwards they were reduced in size sufficiently to retain a part of the virus. This suggests a variation in the size of virus particles. At the other acid reactions the filters were impervious, or nearly so, at the beginning of filtration before the pores were clogged. In these cases, retention of virus by the filters was probably due to strong adsorption.

Fractional Filtration of Virus through Berkefeld "V" Candles

The quantity of virus passing through Berkefeld "V" candles in various fractions of the filtrate and the clogging of pores necessary to retain virus was determined by filtering virus at a 1×10^{-1} dilution in distilled water at pH 5.6. The results are given in table 7.

These data show that it required 70 cc. of the filtering suspension to clog the pores to the extent of retaining a part of the virus. At the beginning of filtration the first fraction of the filtrate was 38 per cent as infectious as the nonfiltered. After 20 cc. had filtered, the succeeding fractions were about as infectious as the nonfiltered juice until 70 cc. had filtered. After this the remaining fractions of the filtrate possessed from 6 to 30 per cent the infectivity of the nonfiltered juice.

Elution of Adsorbed Virus with 0.1 molar Disodium Phosphate

Virus adsorbed to the walls of the filter pores during filtration was eluted by filtering 100 cc. of 0.1 molar disodium phosphate solution through

TABLE 7.—*Virus concentration in successive 10 cc. fractions of Berkefeld "V" filtrate of tobacco-mosaic virus diluted* 1×10^{-1} *in distilled water*

Successive fractions of filtrate	Time to filter	Number of local lesions on 20 beans	Filtration
			per cent
1st 10 cc.	10 sec.	33,000	38.4
2nd 10 cc.	10 "	39,900	46.3
3rd 10 cc.	10 "	82,400	95.9
4th 10 cc.	10 "	93,500	108.8
5th 10 cc.	15 "	85,600	99.7
6th 10 cc.	30 "	122,400	140.1
7th 10 cc.	50 "	96,500	112.4
8th 10 cc.	3.33 min.	27,100	31.5
9th 10 cc.	10 "	5,000	5.8
10th 10 cc.	30 "	7,350	8.5
Unfiltered		85,866	
Check–H_2O		0	

the candles after the nonfilterable débris had been removed with a camel-hair brush. The filtrates were fractionated into 10 cc. portions in order to determine the rate of elution. The débris was suspended in 10 cc. of 0.1 molar disodium phosphate and the liquid filtered through cleaned candles. It was subsequently resuspended 9 times and filtered in order to determine the rate of virus elution from the nonfilterable materials in the original suspension. Estimated virus concentration of the various filtrates is given in table 8 as percentage elution of adsorbed virus.

TABLE 8.—*Elution of adsorbed virus from the capillaries of the filter and from the non-filterable débris with 0.1 molar phosphate buffer at pH 8.5*

Successive 10 cc. fractions of filtrate	Eluted virus	
	From filter	From débris
1st 10 cc.	85.34[a] ± 13.35	81.20 ± 9.10
2nd 10 cc.	9.16 ± 10.45	10.23 ± 5.73
3rd 10 cc.	2.68 ± 4.38	5.61 ± 5.39
4th 10 cc.	1.19 ± 1.01	1.18 ± 1.61
5th 10 cc.	0.77 ± 1.34	0.83 ± 1.55
6th 10 cc.	0.34 ± 1.29	0.31 ± 1.14
7th 10 cc.	0.21 ± 0.67	0.27 ± 1.21
8th 10 cc.	0.14 ± 0.40	0.17 ± 0.74
9th 10 cc.	0.09 ± 0.80	0.11 ± 1.61
10th 10 cc.	0.08 ± 0.87	0.09 ± 0.13

[a] = Per cent elution of adsorbed virus.
± = Probable error.

The quantity of virus eluted was proportional to the amount of virus adsorbed to the filter and to the nonfilterable débris. In the first 10 cc. of liquid, 85 and 81 per cent was released from the filter and from the débris, respectively, while in the 2nd 10 cc., 9 and 10 per cent was eluted. From 5 to 0.08 per cent was eluted in the remaining fractions.

The residue on the candles after washing the nonfilterable débris 10 times was suspended in 100 cc. (the original volume of virus filtered) of 0.1 molar disodium phosphate. The fact that the infectivity of this suspension to beans was very low indicated that a very small amount (0.0002 per cent of the original virus) of active virus remained with this material. The infectivity of a given amount of virus when added to the residue indicated the absence of any inhibiting substance.

Filtration of Virus through Positively Charged Filters

An attempt was made to determine the filterability of virus at controlled hydrogen-ion concentrations through plaster-of-Paris filters, which, according to Kramer (19), are positively charged.

Filters were prepared by molding plaster-of-Paris paste into discs the size of doubled Seitz E. K. filter pads. The paste consisted of commercial plaster-of-Paris to which 20 per cent of magnesium oxide had been added and an equal amount by weight of distilled water. After the discs had dried for a day, they were attached to Seitz filters and 20 cc. of virus (1×10^{-1} dilution) at pH 8.5 and 1.5 forced through under 100 cm. water pressure. After filtration, the discs used at pH 8.5 were washed by passing through 20 cc. of 0.1 molar phosphate buffer at pH 1.5, and those used at pH 1.5 were washed at pH 8.5. Infectivity of the filtrates and washings was determined by rubbing the samples on beans at pH 8.5.

The filtrates and washings were noninfectious. The fact that these filters were impervious to both basic and acid dyes indicates negative and positive areas in the discs. Apparently the positively charged surface adsorbed virus at pH 8.5 and Congo Red, while that negatively charged adsorbed virus at pH 1.5 and Victoria Blue. Negatively charged areas probably originated from impurities in the plaster-of-Paris or in the magnesium oxide.

Filterability of Virus in Suspension with Bacteria

Filterability and relative size of virus and *Bacillus prodigiosus* were determined in the following experiments. A mixture (100 cc.) of virus at 1×10^{-2} dilution and *B. prodigiosus* cells in heavy suspension was adjusted to pH 8.5 with 0.1 molar phosphate buffer and filtered through 6 different filters. The amount of virus in the filtered and nonfiltered suspensions was estimated by inoculation to beans and the number of bacteria per cc.

was determined by plating out a given quantity of the suspensions in nutrient agar. The results from these measurements are given in table 9.

TABLE 9.—*Filterability of tobacco-mosaic virus in a suspension of Bacillus prodigiosus at pH 8.5 in 0.1 molar phosphate buffer*

Filter	Diameter of filter pores	Bacteria per 1 cc.		Virus concentration[a]	
		Unfiltered	Filtrate	Unfiltered	Filtrate
Berkefeld "V"	10 μ	1,500,000	0	47,920	46,640
" "N"	7 μ	"	0	56,960	56,000
" "W"	4 μ	"	0	42,320	44,000
Seitz E. K.	...	"	0	20,480	24,640
Jena sintered glass	16 μ	"	4,000	20,760	30,240
Bechhold-Konig crucible	12 μ	"	3,000	23,920	20,000
Check		0		0	

[a] = Number of lesions on 20 Scotia beans.

Under the conditions of these experiments, filterability of virus was not affected by bacteria in the filtering suspension. Virus passed through all the filters, while bacteria passed only filters with maximum pores 12 μ or greater in diameter. The size of virus particles, according to these results, is considerably smaller than *B. prodigiosus,* which is 0.5 by 1 μ (Bergey, 6).

Ultrafiltration

Particle size of the virus was estimated by ultrafiltration through Bechhold's (1) acetic acid collodion membranes of various pore diameters.

The stock solution for the membranes consisted of 10 grams of soluble cotton (gun cotton) and 2.5 grams of anhydrous potassium carbonate dissolved in 100 grams of glacial acetic acid. Membranes of various pore sizes, as measured by Bechhold's (2) air-water method, were prepared from solutions containing different amounts of collodion. These solutions were obtained by diluting the stock collodion solution with glacial acetic acid. Membranes from solutions containing 10, 7, 4, and 2 per cent of collodion were prepared by coating the inner surface of Jena glass filters with the desired solution and then solidifying the collodion by submersion in distilled water. After washing the membranes for 2 hours, the maximum pore diameter of each membrane was calculated according to the formula, $r = \frac{2Q}{P}$, where r = radius of pores in cm.; Q = surface tension of water-air in dynes/cm. at temperature of measurement (72.5 dynes/cm. at 22° C.); and P = the pressure in dynes/sq. cm. required to force air through the membranes saturated with water. The pressure required to force air through the 10, 7, 4, and 2 per cent collodion membranes was 1,164–998,

1,209–914, 806–702, and 601–587 mm. of mercury, respectively. The maximum pore diameters, therefore, are 0.187–0.218, 0.181–0.238, 0.270–0.311, and 0.363–0.370 microns. The calibrated membranes were used for ultrafiltration with 700 mm. of mercury negative pressure. The Jena glass filters with adhering membranes were attached to suction flasks with Bailey funnels and 10 cc. of virus (1×10^{-1} dilution of a Berkefeld "W" filtrate) filtered through at pH 8.5 and 4.5. Virus held on the membranes was resuspended in 10 cc. of 0.1 molar disodium phosphate. Estimated virus concentration in the various suspensions is given in table 10.

TABLE 10.—*Ultrafiltration of tobacco-mosaic virus through Bechhold's collodion membranes of graded porosity*

Virus	Diameter of membrane pores (microns)	Reaction of medium	
		pH 5.4	pH 8.5
Control (unfiltered)		114,700[a] ± 6,947.35	173,638 ± 18,201.38
Ultrafiltrate	0.187–0.218	1[b]	0
"	0.181–0.238	0	0
"	0.270 0.311	0	24 ± 5.12
"	0.363–0.370	49 ± 24.28	1,296 ± 277.21
Residue	0.187–0.218	130,000 ± 20,235.00	157,600 ± 10,690.15
"	0.181–0.238	151,500 ± 13,017.85	171,000 ± 23,658.76
"	0.270–0.311	49,150 ± 25,113.95	81,000 ± 12,178.09
"	0.363–0.370	17,775 ± 1,838.01	52,700 ± 10,636.19
Check		0	0

[a] = Average number of lesions on 20 beans.
[b] = Probably a contamination.
± = Probable error.

It is apparent from these data that more virus passed through membranes at pH 8.5 than at pH 4.5. At pH 8.5 it just passed through membranes of 0.270 to 0.311 μ maximum pore diameter, but was retained by membranes with pore diameters of 0.187 to 0.218 and 0.181 to 0.238 μ. Assuming that the virus requires pores from 8 to 15 times its size in order to filter through, as Bechhold (3) found for bacteria, the size of the virus particles is estimated to be 0.018–0.038 μ (18–38 mμ[3]) in diameter. The

[3] m μ = millimicron, 10^{-9} meter.

residue recovered from the membranes impervious to virus was as infectious as the original virus sample, while that from the other membranes was about 50 per cent as infectious.

DISCUSSION

Filterability of tobacco-mosaic virus, like that of vaccine virus (Yaoi and Kasai, 34), is influenced by the reaction of the medium. The fact that the virus is completely filterable at pH 8.5 and nonfilterable at pH 1.5 indicates that the particles are negatively charged at pH 8.5 and positively charged at pH 1.5, since the filters (Berkefeld) are negatively charged throughout this pH range. The partial filterability at intermediate reactions suggests that only a part of the particles are negatively charged at these reactions. The electrical charge on the virus, as determined by cataphoresis, has been reported to be negative between the reactions of pH 4.0 and 9.0 by Takahashi and Rawlins (31), and between pH 5.3 and 8.5 by Olitsky and Hoffman (23), but the amount of virus negatively charged was not determined. According to cataphoretic measurements, the isoelectric point of at least a part of the virus is below pH 4.0. Data from the filtration experiments mentioned above suggest that the virus possesses an isoelectric range, the limits of which are below pH 8.5 and above pH 1.5. Associated amphoteric substances with different isoelectric points might impart an isoelectric range. Virus associated with a filterable substance negatively charged at a given hydrogen-ion concentration would be filterable, while that associated with nonfilterable substances or a filterable substance positively charged at the same reaction would be retained by the negatively charged filter. It was found that virus filterable through a negatively charged filter at a reaction of pH 8.5 was retained by a positively charged filter. Kramer (20) reported that virus (pH not given) was held back by filters that retained Congo Red and allowed Victoria Blue to pass through. The fact that the positively charged filters prepared for these studies contained negatively charged areas explains the retention of virus at pH 1.5. Retention of virus in acid media by Berkefeld candles was probably due to adsorption.

Nonfilterability of the virus at pH 1.5 may have resulted from precipitation at this reaction into aggregates larger than the filter pores. This seems improbable, since about 33 per cent of the retained virus was adsorbed within the pores at reactions of pH 5.6, 4.5, 3.0, and 1.5. If precipitation to this extent had occurred, the virus would have remained on the surface of the filter. A part of the virus, however, may have aggregated into nonfilterable particles. It is probable that the virus retained on the surface of the filter was adsorbed to nonfilterable particles in the virus suspension.

Virus infectivity of various fractions of the filtrates at pH 5.6 showed that adsorption of virus to the filter was strong at the beginning of filtration. When the candles were saturated (after filtering from 30 to 40 cc. through), virus was not held back, although clogging of pores continued. This is in agreement with Beijerinck's (5) statement that the first portions of the filtrate (pH not given) were less infectious than later fractions. Apparently after 50 cc. had passed through, clogging reduced the size of pore to the extent of retaining a part of the virus mechanically. The fact that coarse filters ("V") require more suspension for clogging than the fine filters ("W") indicates that pore size is the determining factor in retaining virus by clogged filters.

The adsorbing filter surface at pH 4.5 and 3.0 apparently was not saturated before the clogged pores retained virus. At pH 1.5 adsorption was sufficiently strong to prevent virus passing before the filters were clogged to any extent. At pH 8.5 there was no adsorption of virus or clogging of pores.

Relative elasticity of virus particles and bacteria may account for difference in filterability. Hatschek (14) reported that oil-emulsion particles under suitable pressure were changed in shape during filtration through pores smaller than their diameter, and upon emergence from the filters they assumed the original spherical shape. This explanation of virus filtration seems unwarranted, since the size of the particles determined by other methods approximates that by ultrafiltration. Bechhold and Schlesinger (4) reported the virus to be 50 mμ in diameter by ultracentrifugal analysis, and Waugh and Vinson (33) reported it 10 mμ in diameter by coefficient of diffusion analysis. By ultrafiltration the particle size has been estimated to be 15 mμ by MacClement and Smith (21). Duggar and Karrer (9) reported the virus particles to have a diameter of about 30 mμ. These estimates of the size of virus (10–50 mμ in diameter) are decidedly below the size of *Bacillus prodigiosus* (500 to 1000 mμ, Bergey, 6), but are in the order of magnitude of protein molecules, colloids, and particles of bacteriophage.

SUMMARY

When various dilutions of virus in distilled water were passed through Berkefeld "W" filters the infectivity of the filtrate as compared to the nonfiltered material was greatest in the highest dilutions.

Filterability of virus through Berkefeld "W" candles is determined by the reaction of the medium. At pH 8.5 virus is completely filterable, and at 1.5 nonfilterable. At intermediate pH values it is partially filterable.

Virus suspended in an acid medium is adsorbed to the Berkefeld filter at the beginning of filtration. After the filter surface is saturated with ad-

sorbed materials at pH 5.6, the filtrate is about as infectious as the non-filtered sample until the clogged pores retain most of the virus.

Virus adsorbed to the filters from an acid suspension is readily eluted in phosphate buffer at pH 8.5. About 80 per cent of the virus is released in 10 cc. of buffer.

Filtration of virus at pH 8.5 through Berkefeld "W" candles increased its infectivity 66 per cent.

Virus is separated from *Bacillus prodigiosus* by filtration through Berkefeld and Seitz E. K. filters.

The diameter of the virus particle is estimated to be 18 to 38 mμ.

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EFFECT OF PHOSPHATE BUFFERS ON INFECTIVITY OF TOBACCO-MOSAIC VIRUS[1, 2]

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INTRODUCTION

Studies were made to determine whether various hydrogen-ion concentrations or the presence of salts enhance or depress the infectivity of tobacco-mosaic virus. Since differences in activity greater than about 25 per cent, as estimated by the local-lesion method, are significant, quantitative measurements on infectivity may be made with a fair degree of accuracy. The purpose of this paper is to report results of experiments that were designed to show the effect of different salts at different concentrations and various hyrogen-ion concentrations on infectivity of tobacco-mosaic virus.

REVIEW OF LITERATURE

It has been reported in the literature that a few substances completely inactivate the virus of tobacco mosaic but that many substances have no measurable effect upon it. Relatively few substances are known to increase virus infectivity. Allard (1) reported data that indicated increase of infectivity of tobacco-mosaic virus by sodium sulphate. An increase in apparent virus concentration in the presence of talc and norite was reported by Vinson and Petre (5). They also mention that primary potassium phosphate lowered infectivity, while 0.066 M phosphate buffer at pH 6.5 rendered the supernatant liquid from the lead-acetate-virus precipitate highly infectious. They state that alkaline reactions from pH 9.2 to 9.5 abruptly decreased virus activity. The addition of acid failed to restore the activity of virus that had been completely inactivated above pH 9.2. Lojkin and Vinson (2) reported that the enzymes employed in their studies had no effect upon the virus in fresh untreated tobacco juice, but that trypsin, pancreatin, and papain inactivated the virus of purified preparations. Stanley (4) found that trypsin at certain concentrations protected plants of *Phaseolus vulgaris* L. var. Early Golden Cluster and *Nicotiana glutinosa* L. against infection, while at lower concentrations, it partially inhibited infection. Virus in the virus-enzyme mixture became infective

[1] Presented as part of a thesis submitted to the Graduate Faculty of the University of Minnesota in partial fulfillment of the requirements for the degree of Doctor of Philosophy: granted June 18, 1934.

[2] Published at the Expense of the Rockefeller Institute for Medical Research out of the regular order determined by the date of acceptance of the manuscript.

when the enzyme was destroyed by digestion with pepsin and by exposure to temperatures below the thermal death point of the virus.

METHODS

Virus infctivity was measured by the local-lesion method described by Price (3). Bean plants of the variety Scotia were used in all tests. Twelve days after planting seed, the upper surfaces of the primary leaves of 20 plants were inoculated by rubbing with a bandage-gauze pad moistened with 5 cc. of the virus sample to be tested. Leaves of check plants were similarly rubbed with a pad moistened with water. Because of variation in susceptibility of plants of different ages, all inoculations in a given experiment were made on beans that were planted on the same day and grown under favorable conditions in a greenhouse. Local lesions were counted 5 or 6 days after inoculation.

Johnson's tobacco-mosaic virus 1 was used throughout the studies. Infectious plant sap from macerated Turkish tobacco plants that had been infected for about a month was strained through bandage gauze and frozen immediately. Subsequently, on 2 occasions the liquid was thawed and refrozen. It was thawed and filtered through doubled bandage gauze before use in the experiments.

EXPERIMENTATION

Effect of Various Salts at Different Concentrations on Infectivity

For a preliminary survey of the effect of salts on infectivity, virus was treated with 24 different salts and 2 phosphate buffer solutions at pH 8.0 and 6.5 before inoculation to beans. The following chemicals were used: trisodium, disodium, dipotassium, diammonium, monopotassium, and monosodium phosphate; sodium carbonate; sodium bicarbonate; sodium and ammonium citrate; sodium tetraborate; boric acid; potassium fluoride, chloride, bromide, and iodide; sodium, ammonium, calcium, and aluminum chloride; and sodium, ammonium, magnesium, and aluminum sulphate. Each was employed at 0.01, 0.05, 0.1, 0.5, and 1 molar concentrations. When solubility of the salt permitted, 2, 3, 4, 5, and 6 molar concentrations were used also. Virus at a dilution of 1×10^{-2} in the salt solutions of various concentrations was allowed to stand 1 hour at room temperature. The inoculations were made without changing the salt concentration or reaction of the virus suspension. All plants were washed in running tap water after inoculation to remove the excess salt from the inoculated leaves. Control plants were inoculated with virus at 1×10^{-2} dilution in distilled water.

The results showed that certain concentrations of the dibasic phosphate salts increased virus infectivity. Ammonium, sodium, and potassium di-

basic phosphate at 0.1 molar concentration (pH 7.2, 8.5, and 8.5, respectively) increased infectivity 229, 353, and 411 per cent, respectively. Trisodium phosphate (pH 11.2) inactivated the virus, while sodium carbonate (pH 10.5) greatly reduced its infectivity, but in no case completely inactivated it. The acid salts, aluminum chloride and sulphate, at all concentrations tested greatly reduced or completely inhibited infection. At 0.1 molar concentration these salts reduced infectivity 91 and 99 per cent, respectively. There was no significant effect upon infectivity by the other salts up to 0.1 molar concentration. Above 1.0 molar concentration all salts, soluble to that extent, reduced infectivity. These data show that the dibasic phosphate salts increase the infectivity of virus to beans, and that 0.1 molar concentration is optimum. There was no evidence that valency of the anion or cation influenced infectivity. The effect of monobasic and dibasic phosphate salts and 2 phosphate buffers at pH 8.0 and 6.5 on the number of lesions produced is shown in figure 1.

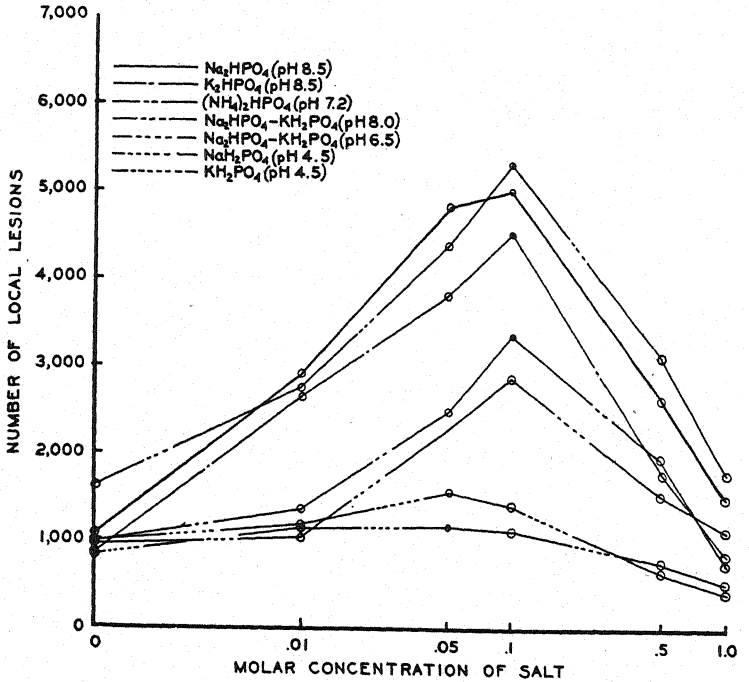


FIG. 1. Graphs showing the effect of 5 phosphate salts and 2 phosphate buffers (pH 8.0 and 6.5) in varying concentrations upon the infectivity of tobacco-mosaic virus at 1×10^{-2} dilution.

Effect of Disodium and Dipotassium Phosphate upon Infection

The effect of disodium and dipotassium phosphate at 0.01, 0.05, 0.1, 0.5, and 1.0 molar concentrations upon infectivity of the virus at dilutions

TABLE 1.—*The effect of disodium and dipotassium orthophosphate upon the infectivity of tobacco-mosaic virus at various dilutions*

Salt	Virus dilution	Control no salt	Molar salt concentration					Increase in infectivity at 0.1 M salt concentration
			0.01	0.05	0.1	0.5	1.0	
								per cent
Na_2HPO_4 (pH 8.5)	1×10^{-0}	6328[a] ± 286.6	7657 ± 296.7	7951 ± 282.6	9341 ± 336.5	7750 ± 243.4	3522 ± 232.7	47
	1×10^{-1}	3491 ± 447.1	5648 ± 551.0	7133 ± 490.3	9065 ± 983.4	6753 ± 480.9	3832 ± 324.4	160
	1×10^{-2}	1098 ± 81.6	2884 ± 509.9	4778 ± 350.7	5028 ± 257.6	2672 ± 254.9	1482 ± 192.2	358
	1×10^{-3}	352 ± 42.4	834 ± 275.1	1007 ± 120.7	1769 ± 369.8	1008 ± 138.9	454 ± 45.1	403
	1×10^{-0}	3549 ± 151.0	4837 ± 390.5	6145 ± 747.3	8487 ± 76.2	5350 ± 583.4	3627 ± 292.0	139
	1×10^{-1}	1463 ± 294.0	2982 ± 248.8	4955 ± 549.7	6250 ± 603.6	3241 ± 120.7	2169 ± 273.1	320
(pH 8.5) K_2HPO_4	1×10^{-2}	885 ± 164.5	2701 ± 126.1	3812 ± 146.3	4522 ± 380.4	1800 ± 29.0	860 ± 101.1	411
	1×10^{-3}	343 ± 11.4	1343 ± 238.7	1823 ± 66.1	2085 ± 707.5	976 ± 18.8	172 ± 6.7	508

[a] = Average number of lesions on 20 beans.
± = Probable error.

of 1×10^{-0}, 1×10^{-1}, 1×10^{-2}, and 1×10^{-3} was determined. The salt-virus mixture was allowed to stand 1 hour before inoculation. Control plants were inoculated with virus at comparable dilutions in distilled water. The results are given in table 1.

The data show that infectivity in all cases was increased by the salts, up to 0.5 molar concentration. In 1.0 molar concentration the virus was as infectious as that in the control. The greatest increase in all cases was at 0.1 molar concentration of salt. There was no significant difference between the effect of the disodium and dipotassium phosphate. Concentration of 0.1 molar disodium of dipotassium phosphate, therefore, is optimum for infection on Scotia beans with virus at dilutions up to 1×10^{-3}.

Effect of Phosphate Buffers at Various Hydrogen-ion Concentrations upon Infection and Virus Activity

Effect of hydrogen-ion concentration upon infection was determined by inoculating the samples of virus of a 1×10^{-2} dilution at reactions of various pH values. Effect of the buffers upon virus activity was measured by exposing virus to various hydrogen-ion concentrations for 1 hour and then adjusting the reaction to pH 8.5 before inoculation. Phosphate buffers at 0.1 molar concentration of salt were used to maintain the desired

TABLE 2.—*The effect of pH upon infection and activity of tobacco-mosaic virus*

pH	Infection at given pH value	Activity of virus after exposure to given pH
Control (5.6)	895[a] ± 60.77	2740 ± 291.31
1.5	239 ± 15.37	2730 ± 186.97
2.0	510 ± 84.04	2450 ± 180.22
3.0	863 ± 24.21	2593 ± 263.72
4.0	1183 ± 98.61	2407 ± 309.39
4.5	1208 ± 90.58	2487 ± 188.59
5.0	1348 ± 140.29	2443 ± 295.49
6.0	1738 ± 136.31	2419 ± 314.11
6.8	2281 ± 294.01	2643 ± 237.49
7.0	2935 ± 80.60	2743 ± 279.58
7.2	3154 ± 137.59	2737 ± 225.82
8.0	3504 ± 50.85	2733 ± 202.61
8.5	3098 ± 81.34	2677 ± 304.94
9.0	1525 ± 41.68	2477 ± 127.41
10.0	1010 ± 69.60	1951 ± 462.16
10.3	919 ± 116.68	1225 ± 85.99
10.5	645 ± 180.83	677 ± 185.55
10.6	264 ± 42.20	124 ± 36.35
10.7	17 ± 1.14	15 ± .87
10.8	0	0
11.0	0	0
11.2	0	0
Check	0	0

± = Probable error.
[a] = Average number of lesions on 20 beans.

hydrogen-ion concentration between pH 1.5 and 11.2. Control plants were inoculated with virus diluted 1×10^{-2} in distilled water. The results are given in table 2.

These data show that reactions from pH 7.0 to 8.5 are optimum for infection on Scotia beans. In more acid or more alkaline media the virus was decidedly less infectious. Inoculations for the measurement of virus infectivity or concentration, therefore, should be carried out at a reaction between pH 7.0 and 8.5. When the virus was adjusted to pH 8.5 before inoculation to beans, there was no appreciable difference in the infectivity of the samples that had been exposed for 1 hour between pH 1.5 and 9.0. Reactions above pH 9.0 reduced infectivity, while those from pH 10.8 to 11.2 inactivated the virus. It appears from these data that virus activity is not changed in 1 hour at room temperature between pH 1.5 and 9.0.

Effect of 0.1 Molar Disodium Phosphate on Dilution Capacity of Virus

Virus infectivity at dilutions from 1×10^{-0} to 1×10^{-8} in 0.1 molar disodium phosphate was compared with virus at comparable dilutions in distilled water. The suspensions were allowed to stand for 1 hour at room temperature before inoculation to Scotia beans. Results of the experiments are given in table 3.

TABLE 3.—*Effect of 0.1 molar disodium phosphate upon the measurement of virus concentration by the local-lesion method on beans*

Dilution value	Virus diluted in	
	Distilled water	0.1 M Na_2HPO_4
1×10^{-0}	7416[a] ± 536.9	9813 ± 664.3
1×10^{-1}	4333 ± 358.8	8306 ± 386.4
1×10^{-2}	1489 ± 80.9	4892 ± 242.1
1×10^{-3}	485.4 ± 60.0	1815.6 ± 191.5
1×10^{-4}	58.2 ± 24.2	567.0 ± 81.6
1×10^{-5}	7.0 ± 1.3	37.0 ± 3.9
1×10^{-6}	0.6 ± .51	3.8 ± .45
1×10^{-7}	0.0	0.4 ± .51
1×10^{-8}	0.0	0.0
Check	0.0	0.0

[a] = Average number of lesions on 20 beans.
± = Probable error.

These results show that 0.1 molar disodium phosphate increased the infectivity of virus at all dilutions that were infectious. At 1×10^{-7} dilution the sample containing salt was infectious, while that without salt was

noninfectious. In no case was infection obtained from the virus at a 1×10^{-8} dilution. The number of lesions produced by virus at 1×10^{-4} to 1×10^{-7} in 0.1 molar disodium phosphate was proportional to the dilution value.

Rate of Virus Inactivation in Alkaline Phosphate Buffers

The rate of virus inactivation in alkaline phosphate buffers was determined. Inactivation was measured by exposing for varying periods of time a 1×10^{-2} dilution of virus to phosphate buffers at pH 9.0, 10, 10.5, 10.6, 10.7, 10.8, 11.0, and 11.2. At the end of the desired exposure, each virus suspension was adjusted to pH 8.5 before inoculation to beans. Control plants were inoculated with a 1×10^{-2} dilution of virus in distilled water. The results are given in table 4.

TABLE 4.—*Inactivation of tobacco-mosaic virus by alkali at room temperature. The reaction was adjusted to pH 8.5 before inoculation to beans*

Virus dilution 1×10^{-2} in 0.1 M phosphate buffer	Time of exposure								
	5 mins.	10 mins.	15 mins.	30 mins.	1 hr.	2 hrs.	3 hrs.	4 hrs.	6 hrs.
pH 9.0					2660[a]	2920	1760	3300	3120
pH 10.0					3300	2780	3220	2080	2420
pH 10.5					1680	1880	1220	68	192
pH 10.6					52	4	4	0	0
pH 10.7					4	0	0	0	0
pH 10.8	368	132	260	36	0				
pH 11.0	20	16	0	0	0				
pH 11.2	0	0	0	0	0				
Control in H_2O									
pH 5.6	2500	2230	2680	2835	2540	2340	2600	3160	2720
Check	0	0	0	0	0	0	0	0	0

[a] = Number of local lesions on 20 beans.

The table shows that virus was inactivated within 5 minutes at pH 11.2, and within 15 minutes at pH 11.0. At pH 10.5 inactivation was slight within 1 hour, and incomplete in 6 hours. Inactivation was complete at this dilution within 1, 2, and 4 hours at pH 10.8, 10.7, and 10.6, respectively. There was little change in virus activity at pH 9.0 or 10.0.

Rate of Virus Inactivation in Acid Phosphate Buffers

The rate of virus inactivation in acid buffers was determined. For these experiments, virus at 1×10^{-2} dilution was exposed for 1, 2, 3, 4, and 6 hours in solutions at various acidities. At the end of the exposure period

the samples were adjusted to pH 8.5 before inoculation to beans. Phosphate buffers more acid than pH 1.5 (acidity of 0.1 molar H_3PO_4) were obtained by adding HCl to 0.1 molar H_3PO_4. The acidity of these buffers is expressed in normality of HCl. Aluminum sulphate at 0.05 and 0.1 molar concentration was included. The control consisted of a comparable dilution of virus in distilled water. The results are given in table 5.

TABLE 5.—*Inactivation of tobacco-mosaic virus by acid at room temperature. The reaction was adjusted to pH 8.5 before inoculation to beans*

Virus dilution 1×10^{-1} in acid media	Time of exposure				
	1 hr.	2 hrs.	3 hrs.	4 hrs.	6 hrs.
in 0.1 M. phosphate buffer at pH 2.0	3120[a]	2600	3140	2760	3140
in 0.1 M. phosphate buffer at pH 1.5	2550	3040	3000	2940	3060
in 0.1 M. H_3PO_4 and 0.01 N HCl	3380	2800	2460	2600	2740
in 0.1 M. H_3PO_4 and 0.1 N HCl	2300	2300	2200	2980	1560
in 0.1 M. H_3PO_4 and 0.5 N HCl	0	0	0	0	0
in 0.1 M. H_3PO_4 and 1.0 N HCl	0	0	0	0	0
in 0.1 M. $Al_2(SO_4)_3$	3540	2940	3020	2860	3200
in 0.05 M. "	2780	2900	3200	3240	3060
Control — in water pH 5.6	3440	1840	1900	1880	3020
Check	0	0	0	0	0

[a] = Number of local lesions on 20 beans.

It is evident from these data that 0.5 and 1.0 N HCl inactivates the virus in 1 hour at room temperature. Apparently slight inactivation occurred in 0.1 N HCl in 6 hours, but none in 0.01 N HCl or at pH 1.5 or 2.0. Aluminum sulphate had no effect upon virus activity when the reaction was adjusted to pH 8.5 before inoculation.

DISCUSSION

Infectivity of tobacco-mosaic virus was found to be influenced by salt and hydrogen-ion concentration. At 0.1 molar concentration, nearly all the salts tested increased infectivity to some extent, while concentrations above 1.0 molar reduced the amount of infection. This reduction in infectivity may have resulted from the precipitation of virus into fewer infective particles or from the action of high salt concentrations upon the inoculated plants. Reduction in the presence of aluminum sulphate was, no doubt, due to the acidity of the solution (about pH .15) and not to the presence of the salts, since the virus possessed its original infectivity when the reaction was adjusted to pH 8.5 before inoculation.

Dibasic phosphate salts in solution at 0.1 molar concentration (pH 7.2 and 8.5) greatly increased infectivity, while monobasic phosphate salts

(pH 4.5) increased infectivity very little as compared to the control, which was at pH 6.5. It appears that the amount of enhancement in infectivity is determined by an interaction of hydrogen-ion concentration and salt concentration.

The influence of salt and hydrogen-ion concentration upon infectivity of the virus may be either an effect on the virus or on the host plant, or both. At present, methods are not available for determining the effect upon susceptibility of the host plant. Apparently, virus is not inactivated in 1 hour at acidities as great as pH 1.5. If inactivation occurs at these reactions, virus activity is quantitatively reversible, since original infectivity of the virus is restored upon adjusting the reaction to pH 8.5 before inoculation. The reduction in infectivity above pH 9.0 and complete inhibition of infection at pH 10.6 in 1 hour is, no doubt, virus inactivation. Complete inactivation in acid or alkali is not reversible. This agrees in general with the result on alkaline inactivation reported by Vinson and Petre (5).

In order to maintain favorable conditions for estimating virus concentration or activity, the reaction of the suspension should be adjusted between pH 7.0 and 8.5 in 0.1 molar phosphate buffers previous to inoculation to beans. Due to the difficulty of counting lesions in excess of 300 per leaf, concentrated samples should be diluted before inoculation.

SUMMARY

Valency of the anion or cation of the salts tested had no measurable effect upon infectivity of tobacco-mosaic virus.

Dibasic phosphate salts at 0.1 molar concentration greatly enhanced virus infectivity.

Optimum reaction for infection is from pH 7.0 to 8.5.

Virus was not inactivated in 1 hour between pH 1.5 and 9.0. At pH 10.6 complete inactivation resulted in 4 hours, and at pH 11.2 in 5 minutes. In 0.5 molar HCl inactivation was complete in 1 hour.

The presence of aluminum sulphate greatly reduced infection, but when the reaction was adjusted to pH 8.5 the original virus activity was restored.

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THE INITIATION OF DOWNY MILDEW OF TOBACCO IN NORTH CAROLINA IN 1934[1]

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INTRODUCTION

Evidence was presented in a previous report[2] that downy mildew of tobacco is initiated as the result of employing the same areas, in successive years, as sites for tobacco seed beds. This evidence was based (1) upon the observation that oospores of the pathogen are present within the tissues of affected leaves of tobacco seedlings, and (2) upon the observation that the disease may appear several weeks earlier in seed beds located on the sites of old beds than on those located on new situations. The fact also was established,[2] as determined by the results of trapping experiments, that sporangia of the organism are carried by currents of air. It also may be recalled that downy mildew spread rapidly throughout the flue-cured tobacco districts of Georgia, South Carolina, North Carolina, and Virginia in 1931, the season of its first appearance, and that it subsequently encroached upon the tobacco-growing areas of Maryland, western North Carolina, Tennessee, and Pennsylvania. The reasonable assumptions have been made that air-borne sporangia were the inocula for the introduction of the pathogen into these areas, that the fungus hibernated in situations where the climate was mild, and that it was reintroduced each season into the more northerly areas. A study of the initiation of downy mildew in North Carolina, in 1934, was, therefore, conducted to test these assumptions, and thus to learn more about the hibernation of the fungus and the source of inoculum for primary infection of tobacco in this State.

METHODS

Apparently no one has been able, as yet, to acquire sufficient knowledge regarding the period of dormacy of the oospores of the downy-mildew fungus and of the conditions that are favorable for germination of the oospores, to utilize such information in a study of the yearly initiation of the disease. To date, our studies along this line have given only meagre

[1] A cooperative investigation by the Departments of Botany and Chemistry, Duke University. The writers wish to express their gratitude for their cooperation to Dr. P. M. Gross, and to the several agricultural teachers and farm demonstration agents and interested growers for their cooperation.

[2] Wolf, Frederick A., L. F. Dixon, Ruth McLean, and F. R. Darkis. Downy mildew of tobacco. Phytopath. 24: 337–363. 1934.

and erratic results; consequently, epiphytological methods have been employed in the present investigation. Accordingly, two procedures have been followed.

(a) Stations were established at which daily observations could be made to determine the date of first appearance of the disease in beds located on the sites of old beds in comparison with those on new situations. These stations (Fig. 1) were located near McDonald, N. C. (Robeson County), and Durham, N. C. (Durham County).

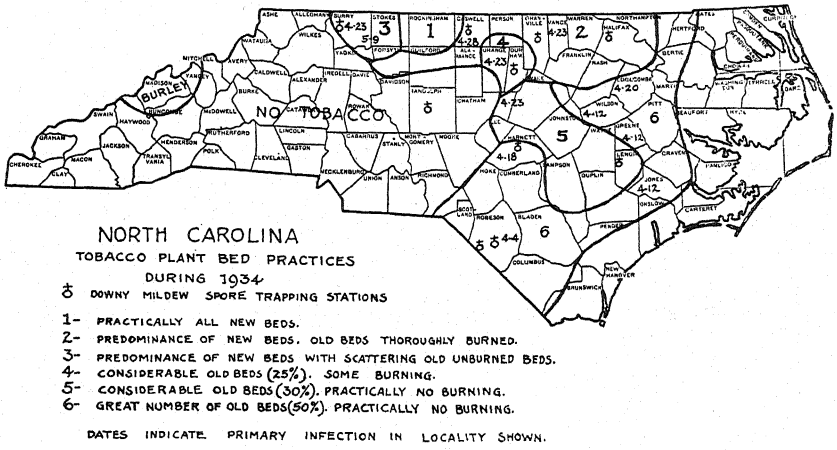


FIG. 1. Map of North Carolina showing the areas in which primary infections were noted, as indicated by the dates; the stations at which spore traps were exposed; and regional practices in seed-bed care.

In making the examinations, the cover was first removed from the bed and then the entire bed was subjected to a systematic and careful scrutiny, as shown by the fact that in one instance a single infected plant was found in a bed with an area of 300 square yards, in another, 3 plants in a bed 200 square yards in area. Spread of inoculum and contamination of the beds was guarded against by attention to such sanitary precautions as frequent changing of shoes and cleansing of hands. It is felt that the frequent examination of the beds did not serve to introduce the downy mildew fungus into any of them, because the disease did not, in any case, appear in the beds that were closely watched prior to its appearance in adjacent, similar, unmolested seed beds.

In order to supplement the observations at these stations, surveys of beds, both of those on old sites and on new sites, were made at certain other points in North Carolina; namely, near Clinton (Sampson County), Snow Hill (Greene County), Wilson (Wilson County), Lillington (Harnett County), Rocky Mount (Edgecombe County), Oxford (Granville County),

Yanceyville (Caswell County), Reidsville (Rockingham County), and Mount Airy (Surry County). The surveys were begun prior to the outbreak of downy mildew in these areas, and examinations were made at frequent intervals until the disease became generally prevalent. The examination and reexamination, both of beds located on old sites and of those on new sites, were made with equally painstaking care. Beds on both types of sites were examined on the same day.

(b) Stations were established in North Carolina to determine, by means of trapping experiments, the earliest occurrence of air-borne sporangia, as related to the earliest occurrence of the disease in that section. These stations were located near Rowland (Robeson County), McDonald (Robeson County), La Grange (Lenoir County), Lillington (Harnett County), Littleton (Halifax County), Farmer (Randolph County), Yanceyville (Caswell County), Dobson (Surry County), Durham (Durham County), and Oxford (Granville County), (Fig. 1). The spore traps consisted of glass plates, 13.3 sq. cm. in area, coated with glycerine and placed 30 inches above the ground level. Four plates, oriented to face each of the cardinal points of the compass, were exposed every 48 hours in each place except near McDonald, Durham, and Oxford. At these places, 8, 10, and 12–24 plates, respectively, were exposed each 2-day period. A total of 1,357 plates were exposed, in which task the teachers of agriculture in the different locations generously cooperated. Examinations were made by first washing off the glycerine and adhering materials, centrifuging the washings, and then examining the sediment microscopically.

RESULTS

(a) *With incidence of downy mildew in beds on old sites and those on new sites:* An idea of practices in one of the areas under observation, with reference to the employment of new and old bed sites and of their relative proximity, can be gained from figure 2, that represents the area near McDonald, N. C.

The growth of tobacco seedlings throughout the State was delayed in 1934 by cold weather. The seedlings were, therefore, smaller than they were at a corresponding date in late March or early April of 1933.

The search for primary infections in 1934 was begun when the tobacco seedlings were less than $\frac{1}{4}$ inch in diameter, and diseased plants were detected when the seedlings had attained a plant spread of $\frac{1}{4}$ to $\frac{1}{2}$ inch. In the majority of instances primary infections were found when sporangia were present on relatively few (1–25) plants in beds 200 to 300 square yards in area. The leaves in contact with the soil were found to be involved first, and at a time when these leaves had not yet become yellow, although yellowing is commonly regarded as the earliest symptom of downy mildew.

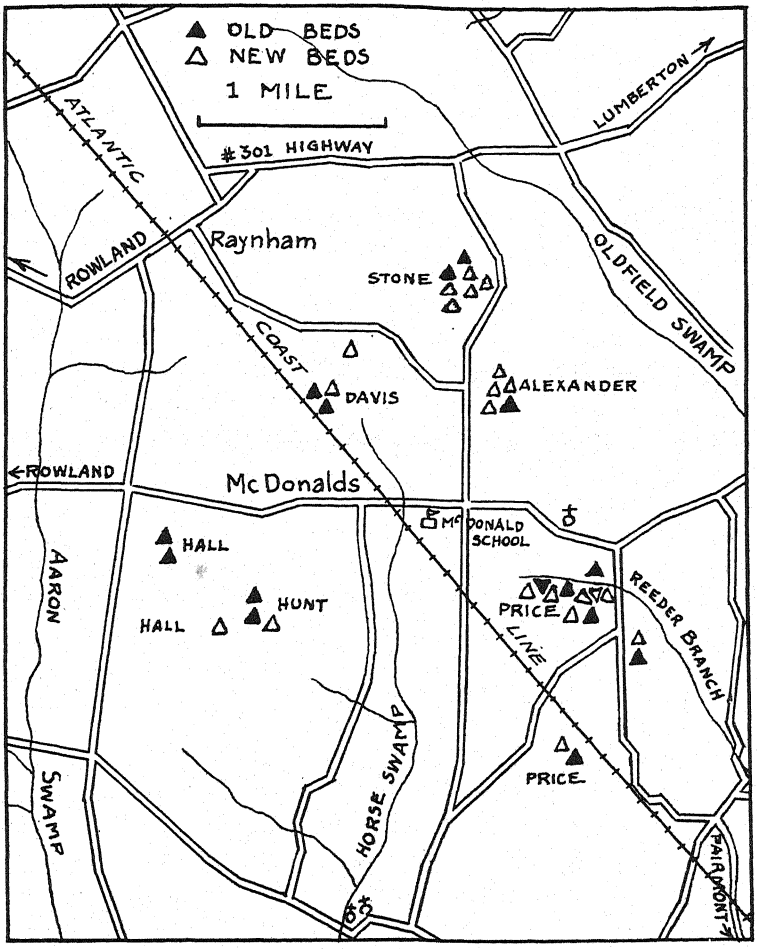


FIG. 2. Map of the environs of McDonald, N. C., showing the distribution of seed beds.

In 1933, the disease was first found somewhat earlier than in 1934, and on seedlings that were considerably larger. The disease had spread, when first seen, to the extent of involving the seedlings in an area a yard or more in diameter. In the light of the present season's observations, there is reason to believe that the disease must have been present, in 1933, 10 to 14 days prior to its discovery. The infections first observed in 1933, therefore, were presumably secondary infections initiated by sporangia from former primary infections in those beds.

Certain data relating to the occurrence of downy mildew in beds located on old sites and in near-by beds on new sites have been assembled in table 1.

The essential features from these data are (1) the distribution of the beds over the flue-cured tobacco-growing area of the State, (2) the approximately equal number and area of beds on the two types of sites, (3) the time of the first occurrence of downy mildew in each locality in beds on each type of site, and (4) the earliest date on which sporangia were entrapped in the air in each locality.

The most striking fact established by this series of observations is that infections occurred earliest, in all cases, in beds located on the sites of beds that were diseased in 1933. No exception to this was noted. Not all primary infections were evident on the same day in any given locality. In Robeson County, for example, an interval of six days occurred between the time of finding the first primary infected bed and the last one, and in Surry County this interval was of 16 days' duration. The fact that the period of primary infections extended in the mountainous Surry County area, over a longer period of time than in the coastal plain region, is explained by the widely differing physiographic features of this mountainous county. To the extent that these features varied locally within Surry County, there was a corresponding variation in the advance of the season, growth of natural vegetation and dates of occurrence of primary infections.

The next important feature is the fact that sporangia were not entrapped in any area until after the downy-mildew fungus was sporulating in old beds in that area. It seems entirely reasonable to infer from these observations, therefore, that the primary infections originated from inocula that had hibernated in these beds. That such inocula consisted of oospores is the logical assumption, as an exhaustive search for overwintering sucker-bearing plants was made and only two such plants were found, neither of which contained hibernating mycelium.

In 1934 downy mildew was first noted in North Carolina on April 4, near McDonald. It was first noted in South Carolina in a bed on an old site near Hemmingway, on the previous day. Attention also should be called to the fact that the initial appearance of the disease in Georgia was about a month earlier, and that it did not become wide-spread in Georgia until about two weeks after it was first found in North Carolina. The disease spread slowly because of the unusually dry, sunny weather prevalent during the downy-mildew season in the tobacco-growing areas surveyed. This slow spread facilitated the distinction between primary and secondary infections.

Another generalization may be made regarding the time of occurrence of primary outbreaks to the northward and westward from McDonald, N. C., as shown in table 1. As the distance from this point increased, the date of the first occurrence of the disease was progressively later, and there was approximately three weeks' difference in time between the first outbreak

TABLE 1.—*Incidence of downy mildew in beds on old sites and in those on new sites in various sections of North Carolina in 1934*

Locality (County)	Beds on old sites					Date when sporangia were first trapped	Beds on new sites			
	No. of beds examined	Area of beds examined (sq. yds.)	No. of beds showing primary infections	Area of primarily infected beds (sq. yds.)	Period in which primary infections were first evident		No. of beds examined	Area of beds examined	No. of beds infected during primary infection period	Period in which infections were first evident
Robeson	30	2,470	9	760	April 4–9	April 7	25	3,280	0	April 16–28
Greene	10	2,350	1	300	April 12	April 20	2	350	0	April 20–28
Wilson	14	2,600	7	1,650	April 12–18	.....	7	1,250	0	April 20–28
Edgecombe	2	150	1	60	April 20	.....	2	260	0	May 2–10
Harnett	11	2,600	7	1,950	April 18–23	None	12	2,925	0	May 2–10
Durham	14	1,870	5	500	April 23–24	May 7	20	3,498	0	May 4–11
Granville	6	1,400	2	750	April 23	April 30	5	2,500	0	May 4–11
Sampson	6	700	0	0	.....	.....	11	2,100	0	May 5–10
Caswell	5	920	1	220	April 23	April 24	8	1,330	0	May 10–17
Rockingham	3	500	0	0	.....	.....	7	550	0	May 12–19
Surry	5	850	4	700	April 23–May 9	May 8	9	1,600	0	May 12
Total	106	16,010	37	6,890	April 4–May 9	April 7–May 8	108	19,643	0	April 16–May 19

in the southeastern portion of the flue-cured area and that in the northwestern one. The differences correspond closely with the seasonal differences between these sections. The earliest outbreaks occurred at the time when flowering dogwood, *Cornus florida,* came into blossom. The disease did not reach epiphytotic proportions, in 1934, in southeastern North Carolina until about 3 weeks later than in 1933, but was only 7 to 10 days later in the central Piedmont section.

It also may be noted that 37 of the 106 beds located on the sites of old beds, or 35 per cent, showed primary infection. This proportion is believed to be representative of conditions throughout the State. At first glance, this proportion may not seem important, but if it be borne in mind that none of the 108 beds located on new sites became diseased until subsequent to the occurrence of the disease in some of the near-by beds on old sites, the importance of primary infection of beds on old sites will become apparent. Certain of the remaining 69 beds located on the sites of old beds became diseased earlier than certain of the beds on new sites, others, coincident with them, and others, after the disease had developed in certain of the beds on new sites. The fact that downy mildew does not occur in all beds sown on old sites prior to its occurrence in beds sown on new sites has led to confusion of opinion among growers and investigators concerning this matter of the influence of site on the occurrence of primary infections. It might be expected that the fungus should survive the winter and appear first in all beds on old sites, instead of in a portion of them. Undoubtedly, the character of the site and the treatment given the seed bed in the interval between crops, as pointed out later in this report, influences the chances of survival of oospores. The fact also should be stressed, as has been stated, that primary infections in beds on old sites do not involve all plants in these beds simultaneously, but a few only. Evidently, a relatively small number of oospores, even under the most favorable natural conditions, survive the winter in any given old bed site. The fact that the remaining 65 per cent of the beds on old sites became infected during the same period as those beds on new sites, and exhibited the appearance of secondary infections, led to the presumption that sporangia were the source of inoculum in these old beds.

The use of old bed sites rather than new ones may be the factor that determines whether the grower will be able to secure a sufficient number of seedlings at the time most propitious for transplanting. Downy mildew in primarily infected beds may cause the death of a large number of the seedlings and inhibit the growth of those that survive. This situation is represented in figure 3, that shows two beds of the same age in the same neighborhood, as they appeared on May 10, 1934. The one on the old site, shown above, was found to be primarily infected on April 23, and the disease soon

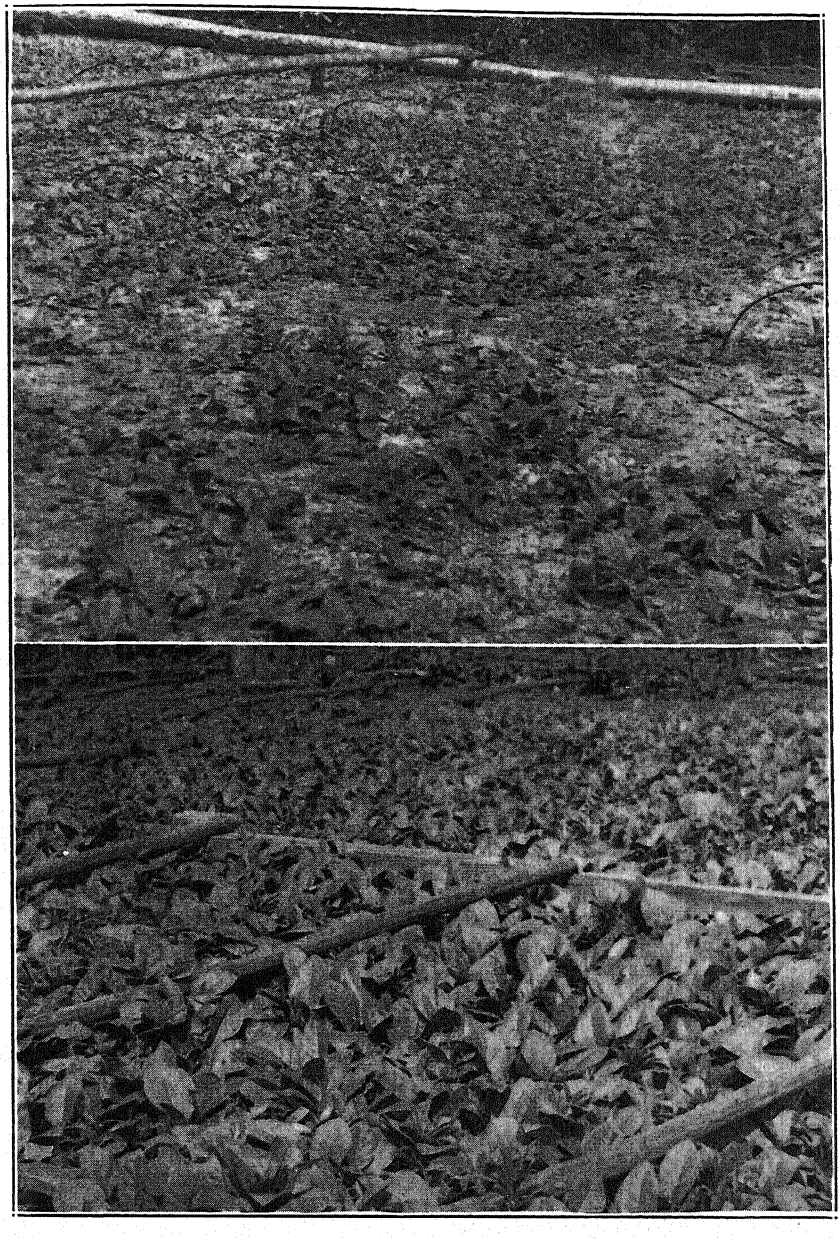


FIG. 3. Seed beds near Durham, N. C., as they appeared on May 10, 1934. The upper one was located on the site of an old bed; the lower one on a new site.

involved all of the seedlings. There was no evidence of disease in the other one on the new site, until May 11. Those that survived in the former did not recover until too late to be used, whereas two lots of seedlings had been drawn for transplanting from the latter before May 11.

(b) *With air-borne sporangia:* The differences in time existing between the primary sporulation in beds sown on old sites and the first occurrence of air-borne sporangia in the vicinity of such beds (Table 1) indicates that the northward progression of downy mildew, in 1934, can not be satisfactorily accounted for by assuming that air-borne sporangia have been introduced from distantly removed southern areas. The data seem rather to indicate that the sporangia borne on primarily infected seedlings serve as inoculum for secondary infections in other seed beds in that same locality. The differences in dates of occurrence of primary infections at the different stations to the northward of McDonald, N. C., correspond with the seasonal differences at these stations, as has been shown. It would appear, therefore, that the wave-like northward progression of the disease might more reasonably be attributed to differences in the time of ocurrence of primary infections, in the several localities, rather than to sporangia that may have been transported to these localities.

Spread of sporangia for short distances has been known to have been accomplished by the agency of man. Evidence, from our observations during the past season, indicates that in some cases the inoculum was spread by the grower. Indiscriminate passage from infected areas to noninfected ones, during weeding, or from one bed to another, resulted in the carriage of sporangia on the hands and clothing of the grower and in subsequent infection. It is also possible that disease-free beds located on old sites, or those on new sites, may become contaminated in this manner.

(c) *With seed bed practices:* The season's observations have emphasized the existence of a variety of practices as to methods of preparation and care of the seed beds and of handling the seed bed sites after the seedlings have been transplanted.

The tobacco-growing sections of the State have been classified as shown in figure 1, according to the prevalent practice in regard to seed-bed care and preparation. While variations occur within nearly all of the sections, a given practice usually was sufficiently widespread to justify a generalized classification. The evidence from observations made in 1933 and 1934 indicates that the number and distribution of old beds in any given locality largely determine the rate of spread of the disease, and, in so doing, modifies the seriousness of the outbreak in that locality. The general situation as to the relationship of the handling of old bed sites and the incidence of primary infection is given in the data compiled in table 2.

TABLE 2.—*Seed bed practices as related to the incidence of primary infection*

Treatment of beds	No. of beds on old sites	No. of beds showing primary infection	No. of beds infected simultaneously with beds on new sites	Primary infections in each class
				(*Per cent*)
No infection during 1933	3	0	3	.....
Burned prior to 1934 sowing	20	4	16	20.0
Not burned prior to 1934 sowing	62	33	29	53.2
Thoroughly burned (4 cords of wood per 100 sq. yds. of bed)	11	1	10	9.1
Slightly burned (brush piles, corn stalks, few pine slabs)	9	3	6	33.3
Abandoned to plants remaining after transplanting. *Not burned* prior to 1934 sowing	25	13	12	52.0
Abandoned to plants remaining after transplanting. *Burned* prior to 1934 sowing	15	2	13	13.3
Cultivated in corn or beans after transplanting seedlings. *Not burned* prior to 1934 sowing	5	2	3	40.0
Cropped with cowpeas between plantings. *Not burned* prior to 1934 sowing	3	1	3	33.3
Covered with wheat straw between plantings. *Not burned* prior to 1934 sowing	10	9	1	90.0
Plowed immediately after transplanting, but not sown in any crop. *Not burned* prior to 1934 sowing	4	4	0	100.0

The data employed in table 2 deal with 85 of the 106 seed beds examined, and present on the sites occupied by beds in 1933. The remaining 21 beds, on old sites, have not been used because their treatment was different from any of the classifications made in table 2. Even though the number of cases in some of the classes is small, the results are believed to be significant. Bed sites that have been "burned" prior to sowing are less likely to show primary infection than those not burned, but this practice cannot be depended upon to completely destroy all inoculum. The covering of the beds with a thick layer of straw immediately after transplanting has been completed and, allowing it to remain undisturbed until time for re-

seeding, is conducive to the preservation of inoculum. The plowing under of the seedlings remaining after transplanting, and reseeding in the following spring, seems also to favor the protection of the inoculum in the soil. This is especially true in beds located in damp, shaded situations.

DISCUSSION

The downy-mildew fungus is known to attack the seed pods of mature plants in the field in Australia,[3] and the seed from such pods have been shown, by microscopic examination, to be invaded by the mycelium of the pathogen. Infection of the seed pods of field-grown plants has not been noted, however, in North Carolina. For this reason infected seed are not believed to constitute a means for the survival of the fungus from season to season here nor for its introduction into seed beds.

Occasional tobacco plants are known to survive the winter in this State. As previously noted, two such plants bearing living suckers were found in the spring of 1934, in spite of the severity of the preceding winter. Both were sectioned and stained but no evidence was found of hibernating mycelium within their tissues. Suckers affected with downy mildew have been found, however, in mid-winter, in southern Georgia. It appears improbable from the observations of the past three years in North Carolina that the survival of the pathogen on old plants constitutes an important factor in its hibernation within the State.

SUMMARY

A study has been made, by epiphytological methods, of the sources of inoculum for the primary infections of downy mildew of tobacco in North Carolina.

The pathogen overwinters in this State, presumably in the oospore stage. There is no evidence that it survives on plants that may have escaped being killed by cold nor that it is seed-borne.

Infections have been found to develop earlier in seed beds located on the sites occupied by seed beds the previous year than on those sown on new sites. These infections were found to occur prior to the time that sporangia could be entrapped from the air in the same locality. The northward march of the disease is attributed to seasonal influences that modify the time of occurrence of primary infections in various localities.

Primary infections were found in 35 per cent of the seed beds sown on the sites of old beds. The downy-mildew fungus was sporulating in beds on old sites from 7 to 19 days prior to the development of the disease in any near-by seed beds located on new sites.

[3] Angell, H. R., and A. V. Hill. Downy mildew (blue mould) of tobacco in Australia. Counc. Sci. Ind. Res. Australia Bull. 65. 1932.

Seed beds sown on the sites of old beds are concluded to constitute primary centers of infection.

Air-borne sporangia or sporangia carried by the grower account for secondary infections.

None of the practices employed in North Carolina with seed beds located on old sites can be depended upon to destroy all of the inoculum in the soil.

The avoidance of utilizing the same site for seed beds in succeeding years is, therefore, of major importance in the prevention of downy mildew of tobacco in the State.

A VIRUS OF CRUCIFERS AND OTHER HOSTS[1]

ISMÉ A. HOGGAN AND JAMES JOHNSON

(Accepted for publication September 22, 1934)

Mosaic of cruciferous plants has been recorded from widely separated districts of the United States (1, 2, 3, 4) and from Europe (5), though whether one or more viruses are concerned can not be determined with certainty from the descriptions published. In the present note a fuller description is given of a crucifer mosaic virus known to affect cabbage, mustard, turnip, and horse-radish. This virus was first brought to the writers' notice in the winter of 1933 by Dr. R. H. Larson of the Department of Plant Pathology, University of Wisconsin, who called attention to some turnip plants showing a conspicuous mottling of the leaves, suggestive of mosaic infection. The plants were among a number being grown for seed production in a cool greenhouse (about 60° F.) and were believed to have contracted the disease in the field during the preceding summer.

By means of artificial inoculation with extracted juice a virus was recovered from two of these plants, which proved to be infectious to various cruciferous and other host species. This virus was transmissible mechanically by plant extract and more readily by aphids, *Myzus persicae* Sulz. and *Brevicoryne brassicae* L. The symptoms on cruciferous hosts appeared to vary considerably according to the temperature and the age of the plants. In general, at about 70–80° F., cabbage, *Brassica oleracea* L. var. *capitata* L., seedlings showed a mild mottling or chlorotic spotting of the leaves (Fig. 1, A, B). Seedlings of mustard, *B. nigra* Koch, and turnip, *B. rapa* L., developed a severe necrosis and yellowing of the foliage, and, later, if the plants survived, a distinct mottling, sometimes accompanied by savoying of the lamina. Leaves and root cuttings of horse-radish, *Armoracia rusticana* Gaertn., believed to be mosaic-diseased, were received in May, 1934, from Kenneth J. Kadow, Illinois Agricultural Experiment Station, Urbana, Illinois, and these proved to contain a similar virus. Plants grown from the root-cuttings showed distinct vein-clearing and a mottling or chlorotic spotting of the foliage.

More striking, however, were the symptoms caused by this virus on tobacco, *Nicotiana tabacum* L. var. Connecticut Havana No. 38, which developed conspicuous brown necrotic lesions on the leaves at points of infection (Fig. 1, C, D, and Fig. 2). These lesions first became visible in

[1] Cooperative investigations of the Wisconsin Agricultural Experiment Station and the Division of Tobacco and Plant Nutrition, Bureau of Plant Industry, U. S. Department of Agriculture.

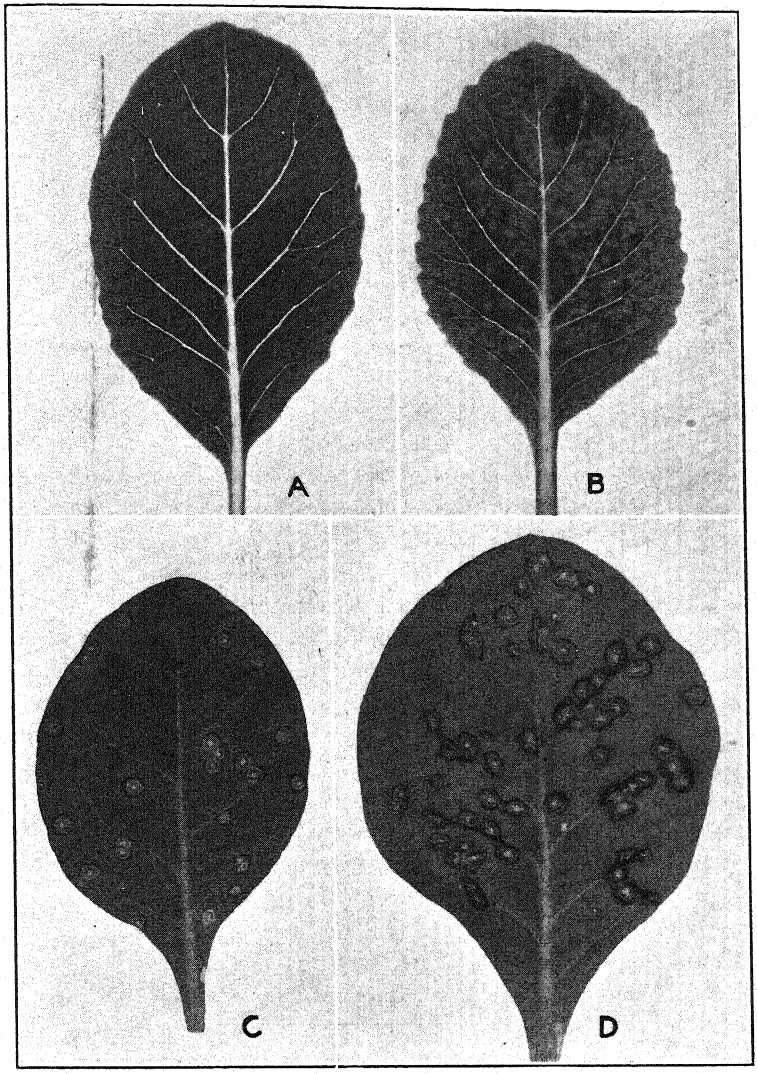


FIG. 1. A. Leaf of healthy cabbage seedling. B. Leaf of cabbage seedling showing mottling caused by the crucifer mosaic virus. C. and D. Leaves of tobacco showing local lesions caused by the crucifer mosaic virus. C. Young lesions produced by *Myzus persicae* from infected cabbage. D. Slightly older lesions produced by rubbing-inoculation from diseased horse-radish.

about 3 or 4 days, and enlarged rapidly up to 5 mm. or more in diameter, usually showing a darker necrotic band at the edge. They sometimes spread a short distance along a vein, but there was no systemic invasion

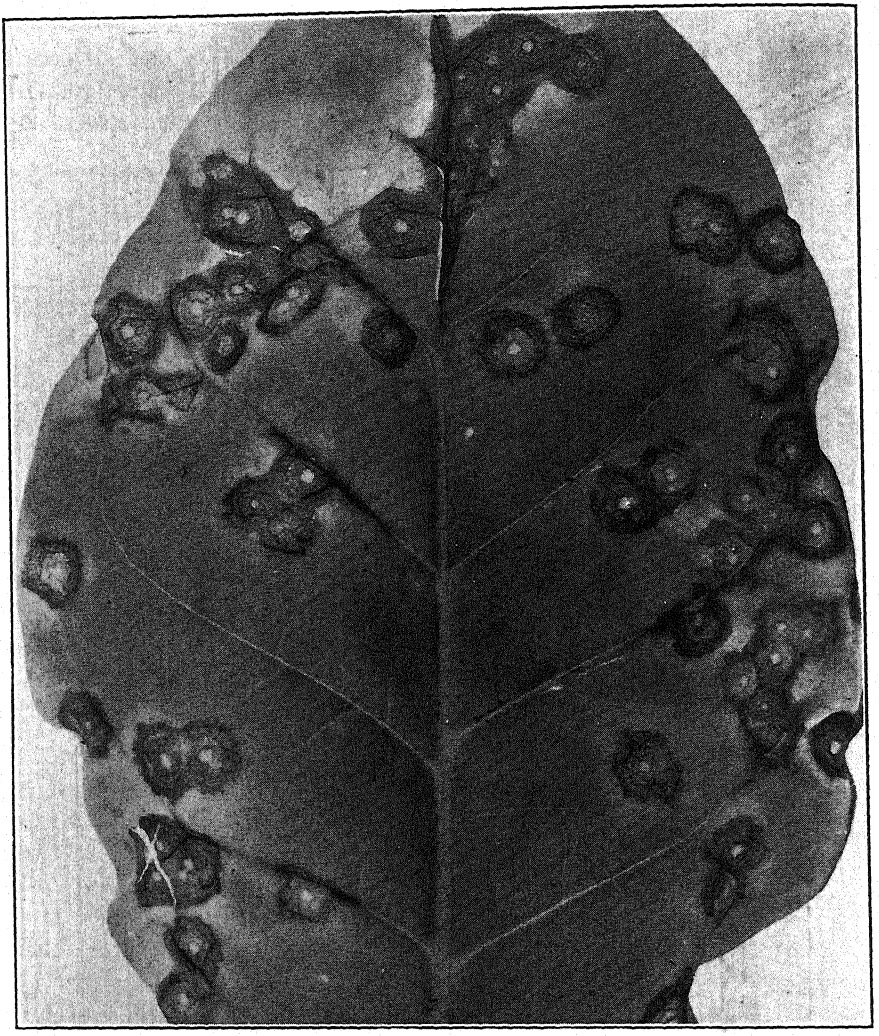


FIG. 2. Old local lesions of the crucifer mosaic virus on tobacco. Approximately $\times\frac{3}{2}$.

of this host. Similar local lesions occurred on the hybrid, *N. tabacum* × *N. glutinosa*, though on *N. glutinosa* only mild chlorotic spots developed, with occasional faintly necrotic rings. Faint chlorotic local lesions developed also on red currant tomato, *Lycopersicon pimpinellifolium* Dunal. On spinach, *Spinacia oleracea* L. var. Bloomsdale, the virus caused systemic infection in the form of a conspicuous yellow spotting and, later, a fine, distinct mottling.

The properties of the virus in extract from cabbage and horse-radish were studied by means of inoculation to tobacco. Each sample of extract,

after treatment, was inoculated by the rubbing method to 2 or 3 leaves of each of 5 tobacco plants, and the number of lesions developing was counted a week or more later. The results of several different tests are presented in summarized form in table 1. It was noted that nontreated extracts from

TABLE 1.—*Properties of the crucifer mosaic virus as determined by the number of lesions formed by rubbing inoculation of extract on tobacco*

Thermal death-point			Tolerance to dilution			Longevity *in vitro*		
Temp. °C. (10 min.)	No. of trials	No. of lesions	Dilution	No. of trials	No. of lesions	Age of extract in hrs. (20–22° C.)	No. of trials	No. of lesions
Nonheated	5	1756	None	5	927	0	4	1219
50	2	115	1 to 10	5	232	12	2	105
52	1	6	1 to 100	5	43	24	4	84
53	3	9	1 to 1000	5	10	36	2	20
54	2	0	1 to 10,000	5	2	48	3	5
55	3	0	1 to 100,000	5	0	72	3	0
Noninoculated controls	5	0	Noninoculated controls	5	0	Noninoculated controls	4	0

horse-radish gave considerably more lesions on tobacco than similar extracts from cabbage, although the point of inactivation of the virus in these tests did not appear to be influenced by the host. The thermal death point for a 10-minute treatment was found to be 54° C. and the tolerance to dilution 1 to 1,000 (in one test 1 to 10,000). The longevity *in vitro* at about 20–22° C., usually lay between 24 and 48 hours, though in one test slight infection was obtained after 48 hours. The longevity *in vitro* was always less than 3 days at the temperature specified.

In a limited study, no inclusion bodies have been observed in association with the disease.

The necrotic local lesions on tobacco are of particular interest in relation to their possible occurrence on this host under field conditions. Tobacco is subject to a variety of leaf spots, the origin of some of which still remains obscure. It is not unlikely that tobacco grown near cruciferous crops or weeds may occasionally become infected with this crucifer virus through the agency of aphids. If so, the connection might not ordinarily be easy to determine, for the virus is not readily recovered from affected tobacco leaves; hence, such leaf spots might be attributed to malnutrition or some other nonparasitic cause.

SUMMARY

A description is given of a crucifer mosaic virus transmissible to various cruciferous and other hosts. Characteristic symptoms are produced on tobacco, consisting of conspicuous necrotic local lesions without systemic infection. It is not known whether this virus is identical with the crucifer mosaic viruses previously reported by other investigators.

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FACTORS THAT INFLUENCE THE FORMATION AND DEVELOPMENT OF MYCORRHIZAL ASSOCIATIONS IN CITRUS ROOTS[1]

H. S. Reed and Thérèse Frémont

(Accepted for publication November 9, 1934)

The conditions for physiological equilibrium between the cells of the Citrus root and their endophytic mycorrhiza appear to depend in large measure upon the chemical and physical nature of the soil and upon the seasonal activity of the tree.

This paper contains the results of studies on this problem in continuation of work initiated at this institution by Rayner.[2]

The material utilized by Rayner and by the writers was collected from orange, *Citrus sinensis,* trees growing on plots on which experiments with fertilizers are being conducted by the Department of Orchard Management of the Citrus Experiment Station. Certain plots have been fertilized annually by definite schedules for the past 7 years; others have received no fertilizers during that time. We have examined roots from: (a) plots that received cover crops and stable manure, (b) plots that received no fertilizers, and (c) plots that received sodium nitrate.

Roots growing in soils that received annually applications of cover crops and stable manure appeared to develop a definite resistance to the invading fungus.[3] There was comparatively little intracellular mycelium in roots from such soils, the hyphae being restricted to short branches that entered the root cells. Once within the root cells, these branches developed ramifications that resemble the "arbuscules" of Gallaud and other investigators.[4] The entrance of the fungus appears to provoke an active response on the part of the root cell, as a result of which the cytoplasm becomes spongy, envelops the arbuscules, and is rich in mitochondria (Fig. 1, A). These changes do not extend to all the cytoplasmic mass, but are confined to the region of the cell containing the invading hyphae, whose contents show modifications similar to those exhibited by the host cytoplasm.

The nature of the cytological changes supports the idea that an active proteolysis operates to digest, first, the intracellular mycelium, then the adjacent cytoplasm of the root cell.

[1] Paper No. 295, University of California, Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, California.

[2] Rayner, M. C. Mycorrhiza in the genus Citrus. Nature (London) **131**: 399–400. 1933.

[3] Reed, H. S., and Th. Frémont. Sur les réactions des cellules des racines de Citrus à l'infection par les mycorrhizes. Compt. Rend. Acad. Sci. (Paris) **199**: 84–87. 1934.

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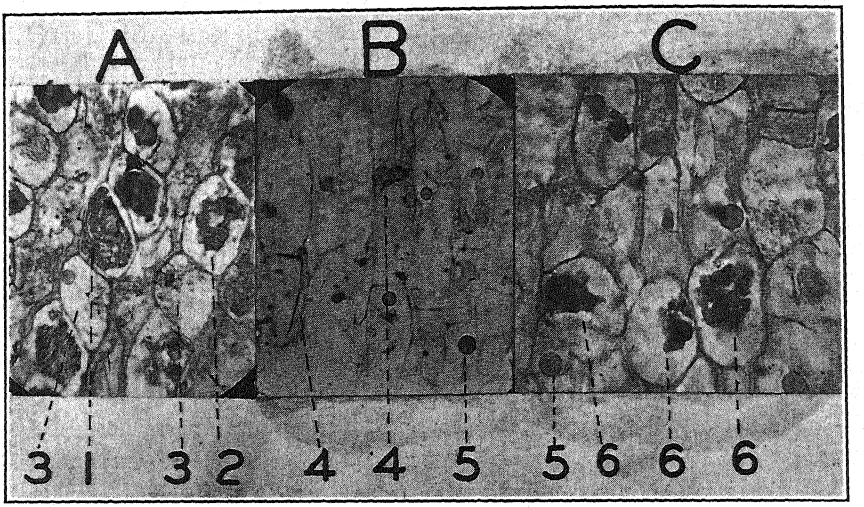


FIG. 1. Photomicrographs of infected orange-root cells showing specific responses to the soil treatments. A. Root from tree that received cover crops and stable manure. 1, cell whose spongy cytoplasm envelops the arbuscule; 2, early stage of digestion; 3, later stage of digestion. B. Root from tree that received no fertilizers. 4, intracellular mycelium of the endophyte; 5, phenolic precipitate. C. Root from tree that received sodium nitrate. 5, phenolic precipitate; 6, hypertrophied cells containing dense masses of endophyte and disorganized cytoplasm.

Roots growing in soils that had received no fertilizers during the preceding 7 years had little power to resist invasion or to digest the intracellular hyphae. In them the mycelium of the fungus seemed to grow as a true parasite. The number of the cells invaded by the fungus was relatively small, the endophyte living principally as intercellular mycelium (Fig. 1, B). The branches that it sent into the cells of the cortical parenchyma were short and sparingly ramified, differing little in their histological characters from the intercellular mycelium. The ends of these branches were often in contact with the nuclei of the cells, as in numerous cases of parasitic Peronosporaceae.

The infection of the root cells, moreover, never provoked an active digestive response on the part of the host, as in the case of roots that received suitable applications of organic and inorganic fertilizers.

The roots of trees that received sodium nitrate as the only fertilizer showed none of the "mycorrhiza cells" in which there was infection followed by digestion. The cortical cells were characterized by their abnormally large size and by an exaggerated reaction to the penetration of the endophytic fungus (Fig. 1, C). Their granular contents absorbed stains with such avidity that it was difficult to recognize anything resembling the

invading hypha, which was the primary cause of the modification of the cell, except where the cytoplasm of the host cell was extremely vacuolated. The trees under discussion that received all their fertilizer in the form of sodium nitrate were unthrifty and suffered severely from the disease known as mottle-leaf. The root cells contained phenolic precipitates which indicated profound disturbances in their metabolism. No evidence of a beneficial association with a fungus could be found.

The relations between the root cells and the fungus also varied with the season when the samples were taken and with the corresponding physiological activity of the tree. The infection of roots occurs principally when the tree is actively growing in the spring months. After growth ceases the mycorrhiza cells gradually disappear. Roots taken from the soil fertilized with suitable forms of organic nitrogen contained an abundance of infected cells only during the period of active, vernal growth. Roots taken from the unfertilized trees, however, contained the endophytic fungus at all seasons of the year. In them, as we have said, the mycelium was intercellular and the reaction of the plant very weak.

In presenting this report we wish to emphasize the importance of the mycorrhizal association as a factor in the metabolism of Citrus-root cells. The relations between the root cell and the endophytic fungus contribute to an understanding of the responses of the trees to nitrogenous fertilizers, and to the conditions favorable for infection by the endophyte.

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PHYTOPATHOLOGICAL NOTES

Breeding millet resistant to smut in North China.[1]—Millet, *Chaetochloa italica*[2] Scribn., is second only to wheat as a food crop in North China. About 22,814,440 acres are devoted to its cultivation, and the annual production amounts to approximately 28,675,548,000 pounds.[3]

As reported by Porter *et al.*,[4,6] kernel smut, caused by *Ustilago crameri* Körn., is a very important disease of millet in China. In the summer of 1932 the disease was very destructive in the vicinity of Kaifeng, Honan, the amount being as high as 25 per cent in at least one field.

Kernel smut can be materially reduced by the use of clean seed and proper seed treatment.[4, 6] Moreover, Porter *et al.*[5] also stated that resistant strains may be readily selected. Since the original resistant strains are no longer available, the present writers have resumed the task of developing resistant varieties of millet to meet the local need.

Altogether, 1,430 head selections were made in the Fall of 1932. Of these 1,273 heads were selected from fields in the vicinity of Kaifeng, while the other 157 heads were selected from farmers' varieties collected from different localities of the province of Honan. Originally, these varieties were tested primarily for high yielding ability and were under test for 4 years. An unnamed variety (No. 48) was secured for checks from Mr. Y. H. Pi, of the cooperative Agricultural Experiment Station of the University of Nanking, and the Southern Baptist Mission at Kaifeng, Honan. This variety yields well and possesses desirable agronomic characters, but its reaction to certain diseases it not known.

The inoculum was obtained from a field in the vicinity of the College of Agriculture, Honan University, Kaifeng, in the fall of 1932. The seeds were thoroughly dusted with the smut spores just before planting.

The planting was made on June 11 to 12, 1933. Each selection was planted to a 15 Chinese-foot[7] row, with a foot between each row. The seed

[1] The authors are greatly indebted to Dr. E. C. Stakman and Dr. J. J. Christensen, of the University of Minnesota, U. S. A., for reading critically the manuscript.

[2] AAMODT, O. S. Varietal trials, physiologic specialization, and breeding spring wheats for resistance to *Tilletia tritici* and *T. levis*. Canadian Jour. of Research, 5: 501–528. 1931.

[3] CHANG, C. C. An estimate of China's farm and crops. 1932.

[4] PORTER, R. H. Seed disinfectants for the control of kernel smut of foxtail millet. Proc. 3rd Pan-Pac. Cong., Tokio, pp. 2103–2107. 1926.

[5] PORTER, R. H., H. K. CHEN, and T. F. YU. Smut resistance in millet. Phytopath. 20: 915–916. 1930.

[6] PORTER, R. H., T. F. YU, and H. K. CHEN. The effect of seed disinfectants on smut and on yield of millet. Phytopath. 18: 911–919. 1928.

[7] A Chinese foot is equivalent to $\frac{1}{3}$ of a meter.

was sown at the rate of 3.5 grams seeds per row. Every tenth row was planted to a check in order to determine variability, if any, due to soil heterogenity, and to make agronomic comparisons. The experiment was not replicated on account of insufficient seeds.

At maturity each row was harvested separately and the percentage of infected heads determined.

To facilitate a detailed examination of the data, the selections were grouped into seven classes on the basis of their susceptibility. Percentages of infection are classified according to Aamodt's scheme, with slight modifications. (Table 1.)[s]

TABLE 1.—*The reaction of 1,430 head selections of millet to kernel smut, Ustilago crameri*

Percentage of smut	Type of reaction	No. of selection	Percentage of selection
0	I	192	13.4
0.1– 5	HR	119	8.3
6.0– 15	R	198	13.8
16.0– 30	MR	379	26.5
31.0– 45	MS	289	20.2
46.0– 60	S	183	12.8
61.0–100	HS	70	4.9

There were striking differences in the susceptibility among the selections. The percentage of infection ranged from 0 to 87 per cent. Although most of the selections developed more than 5 per cent of smut, 192 remained free, while approximately 80 of the selections were more susceptible than the variety used as a control (8.5 per cent). Some of the smut-free selections also possess desirable agronomic characters. Apparently, the varieties of millet commonly grown by farmers in the province of Honan are in heterozygous condition with respect to smut resistance. The data substantiate previous conclusions that selection within a variety is a promising means of isolating desirable smut-resistant strains of millet already adapted to a local community.—CHIH TU and H. W. LI, Honan University, Kaifeng, China.

[s] 0 per cent infection = Immune = I
.1 – 5 per cent infection = Highly resistant = HR
6 – 15 per cent infection = Resistant = R
16 – 30 per cent infection = Moderately resistant = MR
31 – 45 per cent infection = Moderately susceptible = MS
46 – 60 per cent infection = Susceptible = S
61 – 100 per cent infection = Highly susceptible = HS

An Example of Spread of Veinbanding from Potatoes to Tobacco.[1]—Veinbanding, described by Valleau[2] as one of the components of the potato rugose-mosaic complex, often is present in tobacco on the Experiment Station farm at Lexington, Kentucky, and in certain other tobacco plantings. Valleau and Johnson[3] stated that veinbanding is almost never found in tobacco unless it is near potatoes. This virosis usually appears in tobacco prior to handling operations, which indicates that it is transmitted from some other plant by insects. Smith[4] showed that veinbanding (his "y" virus) is readily transmitted from potatoes to tobacco by *Myzus persicae* Sulz. This species is less common on potatoes in Kentucky than the pink and green aphid, *Microsiphum solanifolii* Ashm. It has not been shown that aphids transmit veinbanding from potatoes to tobacco, in the field, but winged forms are not uncommon on tobacco. Even though they do not readily colonize, they may feed enough to cause infection.

In 1934 there was an exceptional opportunity for observing the spread of veinbanding from potatoes to tobacco on the Station farm. Five plots of White Burley tobacco were set the last week in May, near 2 plots of Irish Cobbler potatoes, one of which was planted in the spring and the other about the middle of July. A diagram of the tobacco and potato plots is given in figure 1 and descriptions of the tobacco plots in table 1.

TABLE 1.—*Descriptions of tobacco plots*

Plot No.	No. of Rows	No. of Plants	Position of Rows
1	196	16,722	At right angles to potatoes
2	41	2,477	Parallel to potatoes
3	41	2,375	Parallel to potatoes
4	53	2,812	Parallel to potatoes
5	102	10,210	At right angles to potatoes

The numbers in figure 1 are percentages of veinbanding in groups of 5 rows each of tobacco. The groups of plants in plots 1 and 5 are further divided into thirds, represented by the lines drawn lengthwise of the plots, to better show the decreasing amount of veinbanding as the distance from potatoes becomes greater. In plot 1, in 90 rows opposite potatoes, in the

[1] The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station, and is published by permission of the Director.

[2] Valleau, W. D. The viruses concerned in rugose mosaic of Irish Cobbler potatoes and the weed-host problem. Phytopath. **20**: 135. 1930.

[3] Valleau, W. D., and E. M. Johnson. The relation of some tobacco viruses to potato degeneration. Ky. Agr. Expt. Sta. Bul. 309. 1930.

[4] Smith, K. M. On the composite nature of certain potato virus diseases of the mosaic group as revealed by the use of plant indicators. Proceedings Roy. Soc. Ser. B. **109**: 251–267. 1931.

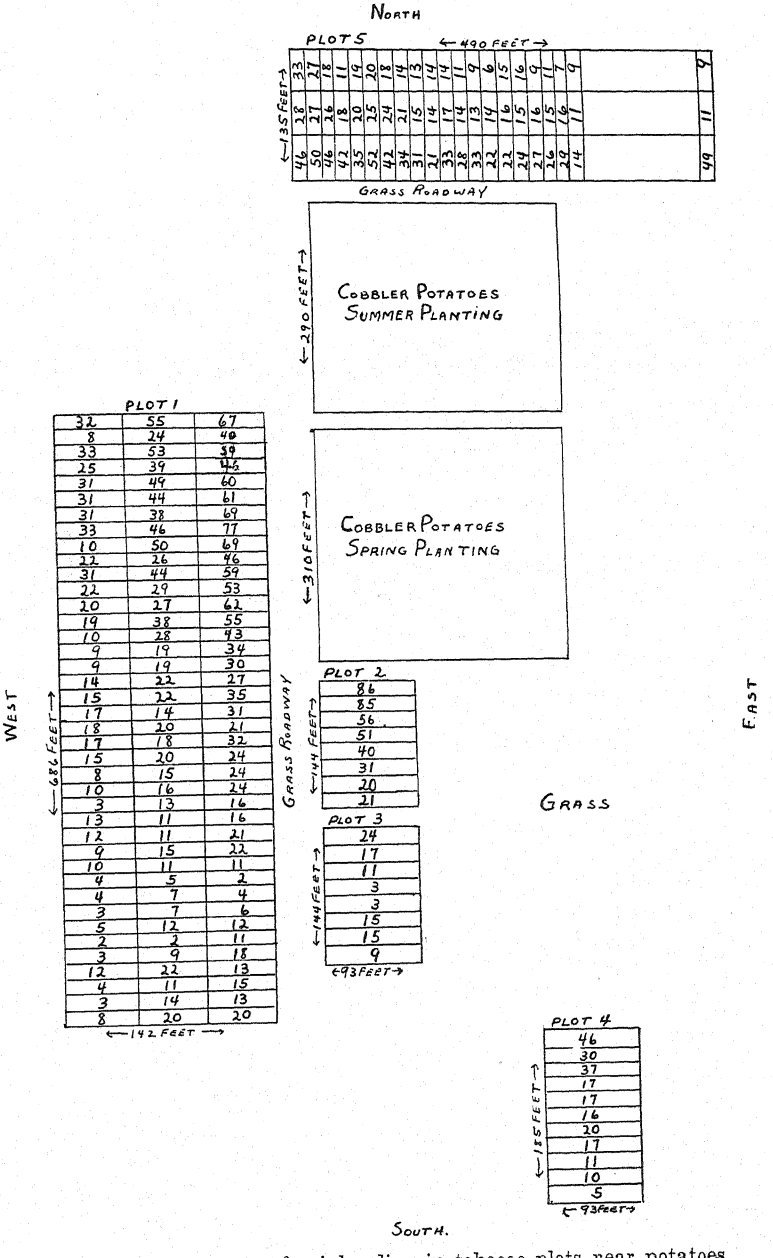


FIG. 1. Percentages of veinbanding in tobacco plots near potatoes.

third nearest potatoes, the middle third, and the third farthest away, 53, 36, and 21 per cent of the plants, respectively, were affected with veinbanding. In the remaining 106 rows, not directly opposite potatoes, the percentages of veinbanding for the corresponding thirds were 18, 13, and 10. In plot 5 the percentages for the corresponding thirds of the plot were 35, 18, and 14.

Observations were made in 4 other tobacco plots on the Experiment Station farm. In one plot of 414 plants, 60 feet from potatoes, 66 per cent of the plants had veinbanding while another plot, 200 feet from the same potatoes, had 19 per cent. Two other isolated plots had 7 and 3 per cent, respectively, the one with the smaller percentage being the greater distance from potatoes. The percentages in all plots show a rapid decrease of veinbanding as the distance from potatoes increases.—E. M. JOHNSON, Kentucky Agricultural Experiment Station.

A root rot of Chinese elms.—A serious root rot of Chinese elms, *Ulmus pumila* L. and *U. parvifolia* Jacq., has recently been discovered on nursery stock grown throughout the Great Plains region. From the diseased roots the fungus *Chalaropsis thielavioides* Peyronel has been consistently isolated. This fungus was originally described by Peyronel[1] as the cause of a disease of lupine and has recently been described by Hammond[2] as the cause of a graft disease of walnuts. Spores of this fungus produce typical rot when placed in Chinese elm roots that have been cut or broken. Fusaria and bacteria are frequently present in the diseased tissue and nematodes have been found consistently associated with *C. thielavioides* in naturally infected roots.

The original infection probably takes place in the seed beds and may spread rapidly in storage or during transit. A grayish white mold-like fungus growth, which appears at injuries or broken places on the roots, is the first observable symptom. The outer root tissues become dark brown to black and are broken down to a slimy mass as the rot advances. Infected plants in the nursery row usually show only the black decayed areas on the roots.

The typical microconidia and macroconidia of *Chalaropsis thielavioides* are produced in abundance, both on decayed roots and in pure cultures Fig. 1). The size of the hyaline to light brown cylindrical microconidia varies considerably in length and thickness but fits in general the dimensions given by Peyronel for his species. These usually are produced from

[1] Peyronel, B. Una nuova malattia del lupino. Staz. Sper. Agr. Ital. 49: 583–596. Tav. II to VI. 1916.

[2] Hammond, J. B. A graft disease of walnuts caused by a species of *Chalaropsis*. (Abstract) Trans. Brit. Mycol. Soc. 19: 158–159. 1935.

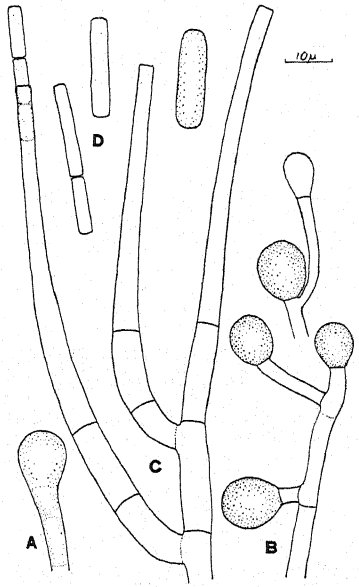


FIG. 1. Conidia and conidiophores of *Chalaropsis thielavioides* from elm. A and B. Macroconidia in various stages of development. C. A group of conidiophores bearing microconidia. D. Microconidia showing variation in size and manner of endogenous formation.

long conidiophores that are hyaline or light brown. The macroconidia are dark, broadly, ovoid to spherical, and borne on short, hyaline conidiophores. Their dimensions also agree with those given by Peyronel. Both types of conidia are very similar to the condidia of *Ceratostomella fimbriata* (E. & H.) Elliott,[3] which always produces a perfect stage; but no perithecial or sexual structures have yet been found for the elm fungus.

Inoculations have definitely proven the pathogenicity of the fungus to *Ulmus pumila* and *U. parvifolia*. Cross inoculations to a number of other tree species have been mostly negative, but there are some indications that the fungus may cause some trouble to several different species, especially as a parasite of cuttings.

Control measures are still in the experimental stage, but it is probable that a chemical dip treatment will be the best means of preventing spread in storage. The fungus appears to live over in the soil. The establishment of new seed beds or the sterilization of old beds with steam or formaldehyde would probably likewise aid in the control of the disease. The elimination of storage should also prevent the spread and rapid development of the fungus.

[3] Andrus, C. F., and Harter, L. L. 1933. Morphology of reproduction in *Ceratostomella fimbriata*. Jour. Agr. Res. 46: 1059–1078. 6 figs., 2 plates. 1933.

The origin of the disease in the Great Plains region is unknown—HOWARD LAMB, ERNEST WRIGHT, and ROSS W. DAVIDSON, Division of Forest Pathology, Bureau of Plant Industry, in cooperation with Emergency Conservation Work and Shelter Belt Project.

Mites on Potatoes.—In the issue of PHYTOPATHOLOGY for July, 1933,[1] is described a potato disease the cause of which is a mite, now definitely identified as *Tarsonemus latus* Banks. The description of the disease as originally published needs slight revision. It now appears that the mites probably were not responsible for the injury to plants in the field. At any rate, no mite injury occurred in the same field in either of the 2 succeeding years, although mites were found on plants in nearby greenhouses. The brittleness of stems and particularly of stolons should not be ascribed to the mites alone. Aside from these slight changes, the injury to potato plants has been found in further observations to be substantially as stated.

In addition to the solanaceous host plants mentioned in the first article, similar severe injury has been observed on beans, *Phaseolus vulgaris,* and on young apple trees, *Pyrus malus,* in greenhouse culture.

Some mild objection has been made (acroamatic) to the implication that it is necessary to graft plants or mutilate leaves in order to transfer the mites. The mites, of course, get about very easily and there is even a suggestion that they may be transferred occasionally and inadvertently by winged creatures. The criticism is valid enough. The explanation is simple. The original outbreak of mites occurred on hybrid plants and for some time it was assumed that this was either another virus disease or else a reaction of these particular hybrid seedlings to acronecrosis or to acropetal necrosis, both of which had become prevalent in the cultures at about the same time and all of which were being studied by the same methods.—DONALD REDDICK.

[1] Reddick, Donald. A potato disease. Phytopath. 23: 622–624. 1933.

BOOK REVIEW

Gitman, L., and E. Boychenko. *Spravochnik po bolezniam novykh loobianykh kul'tur.* [*Manual of diseases of new bast fiber plants.*] 124 pp. 86 figs. Lenin Academy of Agricultural Sciences, Moscow, U. S. S. R. 1934. (In Russian, with English preface and summary.)

Because of the serious drawback caused by fungous parasites to the successful cultivation of the various new bast-fiber plants now extensively grown in the Soviet Union, the Division of Plant Protection of the New Bast Fiber Research Institute of the Lenin Academy of Agricultural Sciences undertook the task of systematizing and codifying all available data bearing on this subject. The authors of this conveniently arranged ready reference brought together in concise form a mass of information hitherto scattered in many different publications or still remaining in the form of unpublished notes or incomplete diagnoses. The object of the little volume as stated in the introduction by Professor N. A. Naumov is to "facilitate the identification of [bast fiber] diseases according to their external manifestation and to the microscopic peculiarities of the pathogen." The manual consists of two major parts. The first part comprises a general discussion of the morbid anatomy of the different parts of the bast fiber plants due to the invasion of bacteria and fungi. In the second part detailed information is given regarding each known malady, and is arranged in a schematic, more or less tabular form. The host plants are arranged in taxonomic order, the plant pathogens are listed in alphabetical order, and the diseases are grouped according to plant parts affected. Disease symptoms and etiology are carefully recorded and literature references are fairly abundant. The many illustrations should prove very helpful.—M. N. Levine, University Farm, St. Paul, Minnesota.

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COMPARATIVE STUDIES OF CERTAIN CULTURES OF PUCCINIA RUBIGO-VERA AND PUCCINIA TOMIPARA ON WILD GRASSES[1]

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INTRODUCTION

The leaf rust of grasses, *Puccinia rubigo-vera* (DC.) Wint. probably is the most composite rust species known from the standpoint of the phenomena of host specialization. Mains (24) distinguished 56 races within this species, one specialized to rye, one to wheat, and 54 to combinations of wild-grass hosts. Physiologic specialization has been most extensively studied in the races on the cereals. Within the rye race, *Puccinia rubigo-vera* sp.f. *secalis* (Eriks. and Henn.) Carleton (*Puccinia dispersa* Eriks.) two physiologic forms have thus far been differentiated by Mains (22). Within the wheat race, *Puccinia rubigo-vera* sp.f. *tritici* (Eriks.) Carleton (*Puccinia triticina* Eriks.) at least 53 physiologic forms are known to exist. These are distinguished by their reaction to a differential set of agronomic varieties of wheat, (25), (15).

In the wild grasses there has not been such a selection and differentiation of varieties as has occurred in cultivated grasses. However, this does not exclude the possibility of the existence within wild-grass species of strains that differ in their reaction to rust.

It was the purpose of the investigations here presented to make a study of several cultures of *Puccinia rubigo-vera,* and of the wild grasses that serve as their hosts, in an effort to obtain some information concerning the extent to which the physiologic specialization of these cultures is comparable to the specialization of the leaf rust of cereals.

[1] Papers from the Department of Botany and the Herbarium of the University of Michigan no. 504.

[2] The writer very gratefully acknowledges the helpful criticism and advice accorded him by Dr. E. B. Mains, under whose supervision these investigations were conducted. He is also grateful to the personnel of the Botanical Gardens of the University of Michigan for the facilities placed at his disposal and for the many courtesies shown.

HISTORICAL SKETCH

The history of *Puccina rubigo-vera* began in 1815, when de Candolle (6) gave the name *Uredo rubigo-vera* to a rust on wheat, in France. Winter (36) in his treatment of the rusts in Rabenhorst's "Kryptogamen-Flora," assigned this species to the genus Puccinia and recognized that it occurred on a number of grasses. With the development of knowledge concerning host restriction, especially with regard to aecial host, various groups have been segregated as species, races, forms, etc.

Thirty-five botanists have proposed 65 names for segregates of *Puccinia rubigo-vera.* Arthur and Fromme (4) have distributed these under 5 rusts: *Dicaeoma Asperifolii* (Pers.) Kuntze, *D. apocryptum* (Ellis and Tracy) Kuntze, *D. procerum* (Dietel and Holway) Kuntze, *D. Cockerellianum* (Bethel) Arthur and Fromme, and *D. Clematidis* (DC.) Arthur. These species differ chiefly in having aecial hosts belonging to different families of plants. Recently, however, Mains (24) united these species and recognized a number of more or less sharply differentiated races, in the resulting composite species, *Puccinia rubigo-vera.* These races were named by Mains (*op. cit.*) and separated by virtue of aecial and telial host combinations and grass host ranges. According to this concept of *Puccinia rubigo-vera,* 86 species of plants, representing 25 genera, and 4 families, serve as aecial hosts for the 56 known physiologic races of this rust species. More than 90 species in 16 genera of grasses serve as uredial and telial hosts. These are distributed in 4 tribes, *viz.,* Agrostideae, 2 races; Avenae, 3 races; Festuceae, 14 races; and Hordeae, 38 races. Still more recently, Arthur (5), in his "Manual of the Rusts in United States and Canada," has recognized this composite species, *Puccinia rubigo-vera,* but divides it into 6 varieties on the basis of aecial and telial host combinations, aided in some cases by spore size and geographic distribution. A complete history of *Puccinia rubigo-vera,* and an organization of the synonymy have been given by Mains (24).

The race restricted to wheat, *Puccinia rubigo-vera,* sp.f. *tritici* (Eriks.) Carleton, has been studied most extensively, especially with regard to physiologic specialization. Mains and Jackson (25) were able to distinguish 12 physiologic forms of the leaf rust of wheat in the United States by the differential reaction of 11 agronomic varieties. Since then, contributions by Scheibe (27) (28), Waterhouse (33), Johnston (14), Johnston and Mains (15), and others have brought the number of known forms up to 53. That some of these forms can be further divided into sub-forms by the use of additional differential wheat varieties has been shown by Waterhouse (33) (34), and Scheibe (28).

No wild-grass race of *Puccinia rubigo-vera* has been studied so inten-

sively; as a result, comparatively little is known regarding host specialization within the wild-grass races.

A few earlier investigators, working with cultures of wild-grass races of *Puccinia rubigo-vera* have noticed differences in reaction between different collections of wild-grass species.

Thus Mains (24, P. 384) found that,

> one collection of *Hordeum jubatum* apparently was resistant to culture Th. 15 of the race *vulgaris* (Table II) and to race *occidentalis* (Table III) and susceptible to other cultures of these races. Likewise one collection of *Elymus virginicus* was apparently resistant to one culture of race *anomala* (Table II) and to one of race *Impatientis* (Table XIII), though susceptible to the other cultures of these races. Though these instances are hardly sufficient to justify conclusions, they suggest that some at least of the wild-grass races may be subdivided through differences in reaction of selections or strains of the various species of grasses.

Mains (*op. cit.* P. 384) further asserts,

> There is no reason to assume that the races with wild-grass hosts differ in this respect from the race *Tritici* on wheat. The last has received extensive study in this regard, but too few collections of both the wild-grass races and their hosts have been studied to justify a comparison.

Mains (23) noted strain differences in resistance and susceptibility in the hosts of *Puccinia dispersa* Eriks. (*Puccinia rubigo-vera* sp.f. *secalis* (Eriks. and Henn.) Carleton), *P. agropyrina* Eriks. (*P. rubigo-vera* sp.f. *persistens* (Plowright) Mains), *P. montanensis* Ellis, and *P. andropogonis* Schw.

Hassebrauk (12), obtained some very interesting results in his studies of the reactions of collections of numerous wild grasses to the several cereal rusts. Besides finding that in many cases the wild grasses are susceptible to the particular forms of cereal rusts that he used, he observed many cases in which a number of collections of the same grass species from different sources behaved quite differently as to rust reaction. For instance, one of his two collections of *Elymus virginicus* was fairly susceptible to form 14 of *Puccinia triticina* Eriks. (*P. rubigo-vera* sp.f. *tritici*), whereas the other was resistant. The two collections had come from widely separated sources.

In this investigation it was thought best to employ a few races of *Puccinia rubigo-vera* and a number of species of grasses. Several collections of each host species, usually from different localities, were obtained in order to study the possibility of reactional differences within grass species.

The investigations of *Puccinia tomipara* are a natural outgrowth of the studies of *P. rubigo-vera* since the former has been considered a race of the latter (3). It differs from *P. rubigo-vera* in producing multicellular telio-

spores. *P. tomipara* was described by Trelease (32) who found it infecting *Bromus ciliatus* L. Arthur (1) considered this to be merely a morphologic variation sometimes met in grass rusts, and when he (1) connected aecia on *Clematis virginiana* L. with telia of the leaf rust type on *Bromus ciliatus,* having normal two-cell teliospores, he therefore used the name *P. tomipara* for this rust. He also used this name again (2) in designating a successful infection of *C. virginiana* from two-cell teliospores from *B. ciliatus.* It is to be noted that these Clematis-Bromus connections were really obtained with typical collections of *P. rubigo-vera.*

Fraser (10) was the first to establish the true aecial connection of *Puccinia tomipara.* Field evidence suggested the connection of aecia on *Thalictrum dasycarpum* Fisch. and All. with leaf rust on *Bromus ciliatus* and species of Elymus and Agropyron. When he inoculated these grasses with aeciospores from *T. dasycarpum,* heavy infection resulted on *Elymus canadensis* L., *E. virginicus* L. and *B. ciliatus* with uredia and telia in great abundance. Microscopic examination of the telia ultimately resulting from these inoculations showed those on the *Elymus* spp. to be of the normal two-cell leaf rust type. The teliospores taken from *B. ciliatus,* however, were

> very variable in size, shape and number of cells, only rarely could two-celled spores of the Puccinia type be found, practically all of the spores being three to several celled, some having as many as sixteen cells.

Fraser (10) inoculated *Elymus virginicus, Agropyron tenerum* Vasey, *A. Smithii* Rydb., *A. repens* (L.) Beauv. and *Hordeum jubatum* L. with this Bromus rust but no infection resulted. This rust was clearly *Puccinia tomipara.* Fraser stated that the multicellular character seemed fixed, inasmuch as many collections, both on *Bromus ciliatus* and *B. latiglumis* (Shear) Hitch. (*B. altissimus* Pursh.), showed this character. He considered it best to include the rust under *P. agropyri* E. and E. until further study established its true position. Fraser also stated that it seemed doubtful whether the rust should even be included in the genus Puccinia.

Arthur *et al* (3) considered *Puccinia tomipara* to be a teratologic race, into which category they also put the related species, *P. elymi* Westend., a multicellular form on Elymus, in which the cells of the teliospores are arranged in linear fashion.

Lagerheim (18) erected the genus Rostrupia on the strength of Westendorp's (35) *Puccinia elymi* and Trelease's *P. tomipara,* considering the multicellular character of the teliospores of these rusts to be a good generic character. Since then several rusts have been assigned to this genus by Dietel (7), but Arthur *et al.* (4), in the North American Flora, reduced these species to synonymy insofar as they apply to North American rusts. Accordingly, *P. elymi* and *P. tomipara* are listed by Arthur *et al.* (4) in the

synonymy for *Dicaeoma clematidis* (DC.) Arth. Mains (24), on the other hand, did not include these two grass rusts in *P. rubigo-vera,* and stated that it seemed best, for the time being, to regard them as distinct species until culture work has shown whether the multicellular character is or is not stable. More recently, Arthur (5) in his "Manual of the Rusts in United States and Canada," cites *P. tomipara* and *P. elymi* as synonyms of *P. rubigo-vera.*

MATERIALS AND METHODS

In these studies 111 collections of species and varieties of wild grasses were used; 65 belong to the genus Bromus; 46 belong to the tribe Hordeae, including 20 numbers of 9 species and varieties of Elymus, 7 numbers of 4 species of Agropyron, 17 numbers of 5 species of Hordeum and 1 number of *Hystrix patula* Moench. It will be noted that in many instances the various species are represented by more than a single collection.[3]

Although 20 collections of *Puccinia rubigo-vera* were studied to some extent, only 9 of these received concentrated study and are reported here. One culture of *P. tomipara* also is included. These rust cultures are referred to in the following pages by numbers only.

The aecial hosts of most of the cultures were determined as a result of clues obtained from field associations. Thus cultures 1, 13, and 20 were connected with Thalictrum; 14, 18, 21 with Impatiens; 22 and 23 with Clematis; connections of cultures 8 and 11 were not determined. Herbarium specimens of the various cultures have been deposited in the herbarium of the University of Michigan.

In Table 1 is given a summary of all the field data concerning these cultures of *Puccinia rubigo-vera* and of *P. tomipara.*

The inoculation methods used in the present investigations have been described by Mains (21). Minor modifications are explained below.

On the most susceptible grass for each rust culture, attempts were made to build up a supply of inoculum with which to inoculate a series of grasses. If these "stock cultures" were watered from below for a few days prior to use for inoculation, the urediospores piled up on each uredium, resulting in the accumulation of a considerable supply of inoculum.

In the process of inoculation, in addition to shaking the heavily rusted "stock plants" over the previously atomized grasses, it was found advan-

[3] The numbers appended to the grass collections are the accession numbers assigned to them by Dr. E. B. Mains who was instrumental in obtaining most of them, especially from foreign botanical gardens. Numbers preceded by B. G. are accession numbers of the Botanical Garden of the University of Michigan. Acknowledgement and thanks are due Dr. J. R. Swallen, of the Smithsonian Institution, and to Prof. J. H. Ehlers of the Department of Botany of the University of Michigan, for checking the identification of the grasses used.

TABLE 1.—*Cultures of Puccinia rubigo-vera obtained from Michigan, Indiana, Ohio and Kansas and of P. tomipara from Michigan*

Culture	Hosts in Field		Where Collected	Collector
	Aecial	Telial		
		Puccinia rubigo-vera		
1	*Thalictrum*[1] *spp.*	*Agropyron repens*	Ann Arbor, Mich.	G. W. Fischer
8	undetermined	*Agropyron repens*	Angola, Ind.	G. W. Fischer
11	undetermined	*Elymus virginicus*	Manhattan, Kansas	C. O. Johnston
13	*Thalictrum dioicum*	*Agropyron tenerum*	Pinckney, Mich.	G. W. Fischer
14	*Impatiens biflora*	*Elymus virginicus*	Ann Arbor, Mich.	G. W. Fischer
18	*Impatiens biflora*	*Elymus virginicus*	Adam's Mills Ohio	E. B. Mains
21	*Impatiens biflora*	*Elymus virginicus* *Elymus striatus*	Ann Arbor, Mich.	G. W. Fischer
22	*Clematis virginiana*	*Elymus virginicus*	Marquette, Mich.	E. B. Mains
23	*Clematis virginiana*	*Bromus ciliatus* *Bromus altissimus*	Pinckney, Mich.	G. W. Fischer
		Puccinia tomipara		
20	*Thalictrum dasycarpum, T. dioicum*	*B. ciliatus* *B. altissimus*	Ann Arbor, Mich.	G. W. Fischer

[1] Determined by inoculation experiments only

tageous to draw these back and forth over the wet grasses very much after the manner in which one would use a paint brush. A more satisfactory distribution of inoculum was effected in this manner than by any other method tried. After inoculation the whole was then incubated 12 to 24 hours under 2 or 3 layers of wet muslin. When uncovered, the grasses were always wet with condensed moisture, thus simulating dew in the field.

Uredia usually began to appear in 8 to 10 days on the most susceptible grasses, but data were not taken until at least 2 weeks after inoculation in order to allow ample time for full expression of reaction. A card file of the grasses proved to be convenient when taking data.

In order to avoid contaminations of one rust culture with another, each was kept, together with its individual series of grasses, in a separate compartment of a portion of the greenhouses especially constructed for rust and mildew investigations at the Botanical Gardens of the University of Michigan.

For inoculation purposes seedlings of the grasses were used, for, in

this stage of growth, the hosts would naturally be in a vigorous condition and a more reliable rust reaction would be secured. Grasses to be inoculated were grown in 2½-inch pots, with 5 to 15 seedlings in each, and were ready for inoculation after 6 or 7 weeks. It is recognized that, in some cases at least, susceptibility reported here for grasses in the seedling stage does not necessarily imply a similar susceptibility in the field or when mature. It has been noted by previous investigators of grass and cereal rusts, notably Johnston and Melchers (16), and Stakman and Piemeisel (30), that certain varieties or strains of the host plant might show a high degree of susceptibility in the seedling stage, but be highly resistant at heading time.

Although it was often found that the types of reaction of the wild grasses to leaf rust are not so clearly defined as are those to the leaf rusts of cereals (a fact also reported by Mains (24)), the symbols used in designating such reactions on cereals have, nevertheless, been found convenient. They are those employed by Stakman and Levine (29) for stem rust, *Puccinia graminis,* and by Mains and Jackson (25) in their studies on the leaf rust of wheat, modified slightly for adaptation to the present studies as follows:

0. Highly resistant ..... No uredia produced; various degrees of flecking produced in some cases, entirely absent in others.
1. Very resistant ..... Uredia few, very small, often abortive; in some cases accompanied by pronounced necrosis; necrotic spots often without uredia.
2. Moderately resistant ..... Uredia better developed than in the preceding; necrotic spots present but less pronounced and not without uredia.
3. Moderately susceptible ..... Uredia well developed, of moderate size; never accompanied by necrotic areas.
4. Very susceptible ..... Uredia relatively large, well-developed, and capable of copious production of urediospores.

The method used in inoculating aecial hosts is the standard one whereby the overwintered telia, under the proper conditions of temperature and moisture, are suspended over the plants to be inoculated. For the most part the plant cover recommended by Mains (21), consisting of a wooden "house" with a glass "roof," was used. The telia of the rust cultures were overwintered in loose cheese-cloth bags or loosely wrapped in mosquito netting. In the spring, as soon as the teliospores showed sufficient germination, inoculations of the aecial host were begun.

RESULTS

Cultural Experiments with *Puccinia rubigo-vera*

Culture 1. On Agropyron repens and Thalictrum spp.—This culture was collected on couch or quack grass, *Agropyron repens,* at the Botanical

Garden of the University of Michigan, and represents a leaf rust that is very prevalent throughout the lower peninsula of Michigan.

Culture 1 was especially adapted to greenhouse study. It was easily propagated in the uredial stage on either seedlings of quack grass or on plants grown from potted rhizomes from the field. Unlike most of the other rust cultures studied, culture 1 produced telia in very scant quantities.

This culture of leaf rust of *Agropyron repens* is very like that found in England on the same host and described by Plowright (26) as *Puccinia persistens,* or the rust designated by Eriksson and Henning (9) as *P. dispersa* f. *agropyri,* and that Eriksson (8) later raised to specific rank as *P. agropyrina.* Mains (24) considered that these names all refer to one race of *P. rubigo-vera.* This he designated as *P. rubigo-vera* sp.f. *persistens* (Plowright) Mains.

Inoculations of a series of grasses during the past two years have given the following results:

DISTINCTLY SUSCEPTIBLE (reaction type 3–4): *Agropyron dasystachyum* (Hook.) Scrib. (1656), *A. repens* (L.) Beauv. (1467, B.G. 13007), *A. tenerum* Vasey (1484, 1659), *Elymus glaucus* Buckley (1539), *Hordeum gussoneanum* Parl. (817, 1393, 1421, 1457), *H. jubatum* L. (1647), *H. murinum* L. (1548).

MODERATELY SUSCEPTIBLE (reaction type 2–3): *Elymus canadensis* L. (1640), *E. virginicus* L. (1793), *Hordeum gussoneanum* (1291, 1433), *H. murinum* (1510), *H. pusillum* Nutt. (1650).

DISTINCTLY RESISTANT (reaction type 0–2): *Agropyron caninum* (L.) Beauv. (1466, B.G. 12770, 803), *Elymus glaucus* (1482), *E. robustus* (S. and S.) Mack and Bush (1642), *E. striatus* Willd. (1645), *E. striatus arkansanus* (S. and B.) (Hitchc. (780)), *E. virginicus* (765, 842, 1475, 1476, 1538), *E. virginicus glabriflorus* (Vasey) Bach. (1646), *E. virginicus submuticus* Hook. (793), *Hordeum murinum* (743, 785, 1290, 1434, 1458), *H. nodosum* L. (1509), *Hystrix patula* (1477).

Plowright (26) showed that the leaf rust of *Agropyron repens* in England produces its aecia on *Thalictrum flavum* L. and possibly *T. minus* L. Treboux (31) reported having connected aecia on *Clematis Pseudoflammula* with uredia and telia on *A. repens.* Mains (24) reported aecia having been produced on *T. delavayi* Franchet, *T. flavum* and *T. minus* from telia of the leaf rust of *A. repens.*

In the present experiments, overwintered telia of *Puccinia rubigo-vera* on *Agropyron repens* suspended over species of Thalictrum in an inoculating chamber, resulted in the production of pycnia and aecia on *Thalictrum minus, T. glaucum, T. flavum* and *T. fendleri.* No infection resulted on *T. dioicum* L., and *T. polygamum.* Similar inoculations of *Clematis virginiana* and *Impatiens biflora* Walt. yielded negative results.

Jackson and Mains (13) pointed out the evident relationship of the leaf rust of *Agropyron repens* to the leaf rust of wheat, emphasizing the fact that they have similar aecial hosts and are morphologically similar. The cultural behavior of the former in the greenhouse is strikingly like that of the latter.

Culture 8. On Agropyron repens (Aecial Host Undetermined). This culture obviously is related to Culture 1, but is physiologically distinct. It was collected at Angola, Indiana, on *Agropyron repens.* No telia were present, and since there was only a very scant production of these sori in the greenhouse, no attempt was made to determine aecial-host connections.

Inoculations of a series of grasses with culture 8 have yielded the following results:

DISTINCTLY SUSCEPTIBLE: *Agropyron repens* (1467), *Elymus canadensis* (795, 1305, 1366, 1537), *E. glaucus* (1482, 1539), *Hordeum gussoneanum* (817, 1393, 1421, 1433, 1457), *H. murinum* (1548).

MODERATELY SUSCEPTIBLE: *Agropyron tenerum* (1489), *Elymus virginicus* (1475), *Hordeum murinum* (1510).

DISTINCTLY RESISTANT: *Agropyron caninum* (803, B.G. 12770, 1466), *A. repens* (B.G. 13007), *Elymus canadensis* (843), *E. striatus arkansanus* (780), *E. virginicus* (842, 1476, 1538), *E. virginicus submuticus* (793), *Hordeum gussoneanum* (1292), *H. murinum* (743, 785, 1290, 1434, 1458), *H. nodosum* (1435), *Hystrix patula* (1477).

It will be seen from the above data that cultures 8 and 1 differ chiefly in the marked susceptibility to the former of certain collections of *Elymus canadensis.*

Culture 11. On Elymus virginicus (Aecial Connection Undetermined). This culture came from Manhattan, Kansas, where C. O. Johnston collected it on *Elymus virginicus.* It was easily and readily multiplied on plants of *E. virginicus* in any stage of development, so that an abundant supply of inoculum was produced. Telia were rarely produced and then in such scant quantities that no attempt was made to determine the aecial host connections.

Inoculations of a series of grasses with culture 11 over a period of 2 years have yielded the following results:

DISTINCTLY SUSCEPTIBLE: *Agropyron dasystachyum* (1656), *A. tenerum* (1484, 1659), *Elymus canadensis* (795, 1305, 1366, 1640), *E. glaucus* (1539), *E. robustus* (1642), *E. virginicus* (765, 842, 1475, 1476, 1793), *E. virginicus glabriflorus* (1646), *E. virginicus submuticus* (793), *Hordeum murinum* (1548), *Hystrix patula* (1477).

MODERATELY SUSCEPTIBLE: *Elymus glaucus* (1482), *E. striatus arkansanus* (780), *E. virginicus* (1538).

DISTINCTLY RESISTANT: *Agropyron caninum* (803, 1466, B.G. 12770), *A. repens* (1467, B.G. 13007), *Elymus australis* (1644), *E. canadensis* (843, 1537), *E. striatus* (1645), *Hordeum gussoneanum* (817, 1291, 1393, 1421, 1433, 1457), *H. jubatum* (1647), *H. murinum* (743, 785, 1290, 1434, 1458, 1510), *H. nodosum* (1435, 1509).

From these results it is seen that this culture of *Puccinia rubigo-vera* finds favorable hosts in a rather wide range of grasses. Eighteen collections of 8 species and 2 varieties, representing 4 genera, have proved to be distinctly susceptible.

Culture 13. On Thalictrum dioicum and Agropyron tenerum. On May 28, 1932, the writer found, near Pinckney, Michigan, plants of *Thalictrum dioicum* heavily infected with aeria. A considerable quantity of this material was collected and used to inoculate a series of grasses.

An abundance of plants of *Agropyron tenerum* and *Elymus virginicus,* heavily infected with telia of leaf rust, were collected in the same locality the following September. These were put outside to hibernate, but each host was kept separately.

In the spring of 1933, the overwintered telia on *Agropyron tenerum* were used to inoculate plants of *Impatiens biflora, Clematis virginiana, Thalictrum dioicum, T. dasycarpum, T. flavum,* and *T. glaucum.* Aecia were produced on the *Thalictrum* spp. only. Another set of the same plants were inoculated from the telia overwintered on *Elymus virginicus;* aecia resulted on *Impatiens biflora* only. This Impatiens-Elymus culture was not further studied.

Inoculations with this culture are not complete. However, the series of grasses inoculated gave the following results:

DISTINCTLY SUSCEPTIBLE: *Agropyron tenerum* (1484, 1659), *Elymus canadensis* (795, 1305, 1366), *E. glaucus* (1482), *E. virginicus* (842, 1475), *Hordeum gussoneanum* (1433), *H. murinum* (1548).

MODERATELY SUSCEPTIBLE: *Elymus virginicus* (1476), *Hordeum gussoneanum* (1457), *Hystrix patula* (1477).

DISTINCTLY RESISTANT: *Agropyron caninum* (803, 1466, B.G. 12770), *A. repens* (1467, B.G. 13007), *Hordeum gussoneanum* (817, 1421), *H. murinum* (743, 785, 1434), *H. nodosum* (1435).

Apparently this is the first demonstration of the connection of *Puccinia rubigo-vera* on *Agropyron tenerum* with *Thalictrum* spp. either by culture experiments or field observations.

Culture 14. On Elymus virginicus and Impatiens biflora. This culture of leaf rust originated from a collection made by the writer on *Elymus virginicus,* northwest of Ann Arbor, in September, 1931. Uredia and telia were present, the latter in abundance; both were collected. The uredio-

spores were sown on seedlings of *Elymus virginicus* in the greenhouse, giving rise to uredia in 9 to 10 days. The telia were not successfully overwintered. In the fall of 1932 more telia were collected from the same region. These were overwintered successfully, and good germination was obtained the following spring. This collection was used to inoculate *Clematis virginiana, Thalictrum dioicum,* and *Impatiens biflora;* pycnia and aecia were produced on the last, only.

The greenhouse culture of the rust on *Elymus virginicus* seedlings was multiplied with fair success for a few weeks. However, during the months of November, December, and January, the uredial culture was nearly lost, the uredia being gradually supplanted by telia. It was not until after February that this culture could be multiplied in the uredial stage.

Repeated inoculations with culture 14 of *Puccinia rubigo-vera* yielded the following results:

DISTINCTLY SUSCEPTIBLE: *Agropyron caninum* (1466), *A. dasystachyum E. robustus* (1642), *E. striatus arkansanus* (780), *E. virginicus* (765, 842, (1656), *A. tenerum* (1484), *Elymus canadensis* (795, 1305, 1366, 1640), 1475, 1793), *E. virginicus submuticus* (793), *Hystrix patula* (1477).

MODERATELY SUSCEPTIBLE: *Elymus glaucus* (1539), and *E. virginicus* (1476).

DISTINCTLY RESISTANT: *Agropyron caninum* (803, B.G. 12770), *A. tenerum* (1659), *A. repens* (1467, B.G. 13007), *Elymus australis* (1644), *E. canadensis* (843, 1537), *E. glaucus* (1482), *E. striatus* (1645), *E. virginicus* (1538), *E. virginicus glabriflorus* (1646), *Hordeum gussoneanum* (817, 1291, 1393, 1421, 1433, 1457), *H. jubatum* (1647), *H. murinum* (743, 785, 1290, 1434, 1458, 1548), *H. nodosum* (1435, 1509), *H. pusillum* (1650).

Culture 18. On Elymus virginicus and Impatiens biflora. This culture of leaf rust came from a collection of telia on *Elymus virginicus* made by E. B. Mains, at Adams Mills, Ohio, September, 1931. These telia were overwintered in the usual manner and a good germination of the teliospores resulted. These were used to inoculate potted plants of *Impatiens biflora, Thalictrum fendleri, T. dasycarpum, T. flavum, T. glaucum, Ranunculus acer, Anemone virginiana* and *Clematis virginiana.* Pycnia and aecia developed on *I. biflora,* only, from which infection the aeciospores were sown on seedlings of *E. virginicus* (842, 1475). A vigorous development of uredia resulted. The culture was multiplied and the resulting inoculum oversummered at a temperature of 10° to 12° C. The following October the culture was renewed from these stored urediospores. A fair infection on *Elymus virginicus* resulted. The uredia gradually reverted to telia until, by the middle of December, the uredial culture was lost entirely. Abundant telia, however, were produced and overwintered and the culture was renewed the

following spring (1932) from inoculations on *Impatiens biflora*. Grass inoculations were carried out in early summer.

Inoculations of a series of grasses with culture 18 have yielded the following results:

DISTINCTLY SUSCEPTIBLE: *Agropyron tenerum* (1659), *A. dasystachyum* (1656), *Elymus australis* (1644), *E. canadensis* (795, 1305, 1366, 1537), *E. glaucus* (1539), *E. striatus* (1645), *E. virginicus* (765, 842, 1475, 1476, 1538, 1793), *Hordeum nodosum* (1509).

MODERATELY SUSCEPTIBLE: *Agropyron caninum* (1466), *Elymus robustus* (1642), *E. virginicus glabriflorus* (1646), *Hordeum jubatum* (1647).

DISTINCTLY RESISTANT: *Agropyron caninum* (B.G. 12770), *A. repens* (1467, B.G. 13007), *Elymus canadensis* (1640), *Hordeum gussoneanum* (817, 1393, 1421, 1433, 1457), *H. murinum* (743, 785, 1290, 1434, 1458, 1510, 1548), *H. pusillum* (1650).

Culture 21. On Elymus striatus and Impatiens biflora. Culture 21 of leaf rust came from a collection of uredia and telia on *Elymus striatus* that the writer made 5 miles south of Ann Arbor in October, 1932. The uredia were used to start the culture on plants of *E. striatus* brought back from the locality where the rust was collected. The telia were put outside to overwinter. They germinated well the following spring and were used to inoculate plants of *Impatiens biflora, Clematis virginiana, Thalictrum dioicum, T. dasycarpum, T. glaucum, T. flavum,* and *T. minus.* Pycnia and aecia were produced on *I. biflora,* only. The aeciospores on Impatiens were used to start the culture from which the grass inoculations were made.

Inoculations of a series of grasses with culture 21 yielded the following results:

DISTINCTLY SUSCEPTIBLE: *Elymus canadensis* (795, 843), *E. robustus* (1642), *E. virginicus* (842, 1476, 1793), *E. virginicus* var. *submuticus* (793).

MODERATELY SUSCEPTIBLE: *Elymus canadensis* (1537, 1640), *E. glaucus* (1539), *E. striatus* var. *arkansanus* (780), *E. virginicus* (1475), *Hordeum gussoneanum* (1421), *H. pusillum* (1650).

DISTINCTLY RESISTANT: *Agropyron caninum* (803, 1466, B.G. 12770), *A. dasystachyum* (1656), *A. repens* (1467, B.G. 13007), *A. tenerum* (1659), *Elymus australis* (1644, *E. canadensis* (1305, 1366), *E. glaucus* (1482), *E. striatus* (1645), *E. virginicus* (765, 1538), *E. virginicus* var. *glabriflorus* (1646), *Hordeum gussoneanum* (817, 1291, 1393, 1433, 1457), *H. jubatum* (1647), *H. murinum* (743, 785, 1290, 1434, 1458, 1510, 1548), *H. nodosum* (1509), *Hystrix patula* (1477).

It is interesting to note that the *Elymus striatus* on which culture 21 was collected and later propagated was highly susceptible, while the *E.*

striatus (1645) from Indianapolis, Indiana, was distinctly resistant. Only moderate susceptibility was shown by the variety *arkansanus* (780) of *E. striatus*. Furthermore, culture 21 is the only culture of *Puccinia rubigo-vera* studied (culture 23 on *Bromus* spp., excepted) that had no susceptible hosts in the genera Agropyron and Hordeum.

Culture 22. On Clematis virginiana and Elymus virginicus. Culture 22 of *Puccinia rubigo-vera* was obtained from aecia collected on *Clematis virginiana* by E. B. Mains, at Marquette, Michigan, June, 1933. The aeciospores, sown on *Elymus virginicus* (1475) and *Bromus ciliatus* (1649), resulted in a heavy infection on *E. virginicus,* only. The culture was multiplied twice, and abundant inoculum was obtained to inoculate the usual series of grasses. Inoculation experiments were carried out during the summer.

Inoculations of the following grasses with culture 22 have given the following results:

DISTINCTLY SUSCEPTIBLE: *Agropyron caninum* (1466), *A. tenerum* (1659), *Elymus canadensis* (1305, 1537), *E. glaucus* (1539), *E. robustus* (1642), *E. virginicus* (765, 842, 1475, 1793), *Hordeum jubatum* (1647), *H. nodosum* (1509), *H. pusillum* (1650).

MODERATELY SUSCEPTIBLE: *Hordeum murinum* (1434, 1458, 1510).

DISTINCTLY RESISTANT: *Agropyron caninum* (803, B.G. 12770), *A. repens* (1467, B.G. 13007), *A. dasystachyum* (1656), *Elymus australis* (1644), *E. canadensis* (795, 843, 1366, 1640), *E. glaucus* (1482), *E. striatus* (1645), *E. striatus arkansanus* (780), *E. virginicus* (1476, 1538), *E. virginicus glabriflorus* (1646), *E. virginicus submuticus* (793), *Hordeum gussoneanum* (817, 1291, 1393, 1421, 1433, 1457), *H. murinum* (743, 785, 1290, 1548), *H. nodosum* (1435), *Hystrix pastula* (1477).

Table 2 summarizes the reactions of the 46 collections of species of Agropyron, Elymus, Hordeum, and Hystrix to the 8 cultures of *Puccinia rubigo-vera* described in the foregoing.

Culture 23. On Bromus ciliatus and Clematis virginiana. This culture was obtained accidentally while working with *Puccinia tomipara.* Overwintered telia on *Bromus ciliatus* collected near Pinckney, Michigan, in September, 1932, were being used to inoculate plants of *Thalictrum dioicum* and *T. dasycarpum* (April 29, 1933). A healthy potted plant of *Clematis virginiana* was also placed in the inoculation chamber along with the *Thalictrum* spp.

Pycnia appeared, not only on the *Thalictrum* spp., as expected, but also on *Clematis virginiana,* followed by aecia on both. Thus it appeared as if *Puccinia tomipara* produced aecia on *C. virginiana,* as well as on species of Thalictrum. The aeciospores from *C. virginiana* were used to inoculate

TABLE 2. *Summary of the reactions of 46 collections of wild-grass species to 8 cultures of Puccinia rubigo-vera*

Wild-grass collections	Cultures of *Puccinia rubigo-vera*							
	1	8	11	13	14	18	21	22
	TYPES OF REACTION							
Agropyron caninum 803	0	0	0	0	0	0	0	0
A. caninum 1466	0	0	0	.....	3–4	3	2	3–4
A. caninum B.G. 12770	0	0	0	0	0	0	0	1–2
A. dasystachyum 1656	4	.....	4	.....	3–4	3–4	0	0
A. repens 1467	3–4	3–4	0	0	0	0	0	0
A. repens B.G. 13007	3–4	0	0	0	0	0	0	0
A. tenerum 1484	3–4	3	4	3–4	4	.....	.....	.....
A. tenerum 1659	3–4	.....	3–4	4	0	3–4	0	3–4
Elymus australis 1644	0	.....	0	.....	0	3–4	0	0
E. canadensis 795	0	3–4	4	3–4	3–4	.....	4	0
E. canadensis 843	0	0	0	0–1	0	.....	3–4	0
E. canadensis 1305	0	3–4	4	3–4	3–4	4	0	4
E. canadensis 1366	0	3–4	3–4	3–4	3–4	3–4	0	0
E. canadensis 1537	0	3–4	0	0	0	3–4	3	3–4
E. canadensis 1640	2–3	.....	3–4	.....	3–4	2	3	0
E. glaucus 1482	0	4	3	4	0	.....	0	0
E. glaucus 1539	3–4	4	4	.....	3	3–4	3	3–4
E. robustus 1642	0	.....	3–4	.....	4	2–3	3–4	3–4
E. striatus 1645	0–2	.....	0	.....	0	3–4	0	0
E. striatus var. *arkansanus* 780	0	0–1	3	.....	3–4	3–4	3	0
E. virginicus 765	0	.....	3–4	2	3–4	3–4	0	4
E. virginicus 842	0	0	4	3–4	4	4	3–4	3–4
E. virginicus 1475	0	3	4	3–4	3–4	3–4	3	4
E. virginicus 1476	0	0	3–4	3	3	3–4	3–4	0–1
E. virginicus 1538	0	0	2–3	0	0	3–4	0	0
E. virginicus 1793	2–3	.....	4	.....	3–4	3–4	3–4	4
E. virginicus var. *glabriflorus* 1646	0	.....	3–4	.....	0	3	0	0
E. virginicus var. *submuticus* 793	0	0	3–4	.....	3–4	.....	3–4	0
Hordeum gussoneanum 817	3–4	4	0	0	0	0	0	0
H. gussoneanum 1291	2–3	0	0	.....	0	.....	0	0
H. gussoneanum 1393	3–4	3–4	0	.....	0	0	1–2	1–2
H. gussoneanum 1421	3–4	3–4	0	0	0	0	3	1–2
H. gussoneanum 1433	2–3	3–4	0	3–4	0–1	0	0	0
H. gussoneanum 1457	3–4	4	0	3	0	0	0	2
H. jubatum 1647	4	.....	0	.....	0	2–3	0	3–4
H. murinum 743	0–2	0–1	0	0	0	0	0	0–2
H. murinum 785	0–2	0	0–2	0	0	0	0	2
H. murinum 1290	0–2	0	0–2	.....	0	0	0	0–2
H. murinum 1434	0–2	0–2	0–2	0	0	0–1	0	2–3
H. murinum 1458	0–2	0	0–2	.....	0	.....	0	2–3
H. murinum 1510	2–3	3	0–2	.....	0	0	0	2–3
H. murinum 1548	3–4	4	3–4	3–4	0	0–1	0	0
H. nodosum 1435	.....	0	0	0	0	.....	.....	.....
H. nodosum 1509	0	.....	0	.....	0	3–4	0	3–4
H. pusillum 1650	3–4	.....	0	.....	0	2	3	3–4
Hystrix patula 1477	0–2	0	3–4	3	4	.....	0–1	0

one pot each of *Bromus ciliatus* and *B. purgans;* two other pots of these grasses also were inoculated with aeciospores from *T. dioicum.* Uredia were abundant in both cases. Telia, however, did not appear, until early July

on the grasses inoculated with aeciospores from *T. dioicum,* and about 2 weeks later on the grasses inoculated with aeciospores from *C. virginiana.* Several mounts of the first telia contained the multicellular teliospores characteristic of *P. tomipara;* occasionally a two-cell spore could be found. The telia resulting from aeciospores from *C. virginiana,* however, were all bicellular; the culture clearly belonged to *P. rubigo-vera,* and was designated as culture 23.

It seemed desirable to compare the host range of this Bromus culture of *Puccinia rubigo-vera* with that of *P. tomipara.* Essentially the same series of Bromus species was inoculated with a uredial culture of *P. rubigo-vera* as was used for a similar study of *P. tomipara.*

Inoculations of a series of Bromus species have given the following results:

DISTINCTLY SUSCEPTIBLE: *Bromus purgans* (1289), *B. ciliatus* (1649), *B. altissimus* (1795).

DISTINCTLY RESISTANT: *Bromus adoensis* Höchst. (1331, 815), *B. arduensis* Dum. (1332), *B. arvensis* L. (708, 1297), *B. brizaeformis* F. and M. (1442), *B. commutatus* Schrad. (716, 771), *B. hordeaceus* L. (719, 1508), *B. japonicus* Thumb. (1347), *B. kalmii* Gray. (1541), *B. macrostachys* Desf. (797, 714, 705, 1446), *B. madritensis* L. (713), *B. maximus* Desf. (1427), *B. rigidus* Roth. (1384, 1452, 1501, 698, 1505), *B. rigidus* var. *gussonii* (Parl.) C. and D. (1506, 1333), *B. rubens* L. (1502), *B. sterilis* L. (701, 1503), *B. squarrosus* L. (1450), *B. tectorum* L. (1547, 768, 1545, 712), *B. uniloides* (Willd.) H.B.K. (1507, 1451, 718, 750).

Comparative Morphology of the Urediospores and Teliospores of Eight Cultures of Puccinia rubigo-vera. Attempts to discover morphological differences between races or between physiologic forms within composite rust species have been made by few investigators. Levine (19) has shown that there are significant morphological differences between the spore forms of the 5 races of *Puccinia graminis* that he studied and that these races can, therefore, be distinguished from one another if a sufficiently large number of spores are measured. The same author (20) later showed that there are also slight but constant differences between the spore forms of different physiologic forms within the race *tritici* of *P. graminis.*

Mains (24) noted a certain amount of variation in the size of the urediospores and teliospores of some races of *Puccinia rubigo-vera.* In some cases such variation is quite marked, and this fact accounts for the tendency to separate certain groups of races as species. Mains (24), however, studied spore sizes of most of the races of *P. rubigo-vera* and stated that many races with aecia on Thalictrum did not differ with respect to urediospore size from the races on Impatiens, but that the latter are somewhat outstanding, as a group, for small size of the teliospores. He showed further that cer-

tain races having aecia on a variety of hosts, do not differ to any appreciable extent in teliospore size, but that a few other races with different aecial hosts do show appreciable differences. In general it was concluded that there is too much intergradation of the races of this rust to permit their being distinguished morphologically with any degree of certainty.

In connection with the physiological studies of the cultures of *Puccinia rubigo-vera* used in the present investigation a study of the comparative morphology of the urediospores and teliospores also was made. Only spores produced in the greenhouse, on the most susceptible hosts, and under uniform favorable cultural conditions were used.

For this study of the comparative morphology of 8 cultures of *Puccinia rubigo-vera,* the urediospores, being so nearly globoid, were measured for diameter only. The teliospores, of course, were measured in two dimensions. The length of the teliospore was considered to be that distance from the distal end to the septum separating the spore from the pedicel; the widest part was assumed to represent the diameter of the spore, exclusive of the thickened apex, which is sometimes wider than any other portion. One hundred and fifty urediospores and the same number of teliospores of each rust culture were measured. These were selected at random from a mount in lactic acid, (to render the germ pores more distinct and also to swell the spores to normal size). Only unquestionably mature spores were measured, the measurements being made to the nearest micron.

The data obtained from these measurements are presented in tables 3 to 5. They show rather marked differences between some of the cultures, especially if one compares their mean measurements. Table 3 shows the results obtained from the measurements of the urediospores of the 8 cultures. The range in diameter for the species as a whole, as represented by

TABLE 3.—*Frequencies of the diameters of urediospores of 8 cultures of Puccinia rubigo-vera*[a]

Culture	Classes according to spore diameter									Mean diameter
	15 μ	16 μ	17 μ	18 μ	19 μ	20 μ	21 μ	22 μ	23 μ	μ
1				2	2	40	47	57	2	21.07
8				2	1	38	58	42	9	19.90
11			6	72	51	21				18.58
13			2	25	30	60	24	9		19.70
22	2	16	83	41	13					17.21
14	7	16	74	48	5					17.18
18			1	14	19	92	21	3		19.84
21				42	40	62	2	2		18.94

[a] One hundred and fifty spores of each culture were measured.

TABLE 4.—*Frequencies of the diameters of the teliospores of 8 cultures of Puccinia rubigo-vera*[a]

Culture	Classes according to spore diameter														Mean diameter
	8 μ	9 μ	10 μ	11 μ	12 μ	13 μ	14 μ	15 μ	16 μ	17 μ	18 μ	19 μ	20μ	21 μ	μ
1			1	2	12	16	39	34	15	15	2	10	4		14.94
8			1	0	9	8	44	36	23	20	2	5	2		14.98
11					2	5	28	53	25	13	13	8	3		15.62
13				4	22	18	45	27	20	7	3	2	1	1	13.26
22			3	5	33	28	38	23	7	7	2	3	1		13.77
14			3	2	26	24	45	25	13	7	2	2	1		14.01
18	12	19	36	33	31	13	5	1							10.77
21	14	15	50	26	25	14	4	2							10.67

[a] One hundred and fifty spores of each culture were measured.

these 8 cultures is 15–23 μ; yet only 2 of the cultures (14 and 22) produced any spores that fell within the 15 μ class and only 2 (1 and 8) had any within the 23 μ class, insofar as this could be determined by measuring 150 spores. The modes for urediospore diameter vary from 17 μ, for cultures 14 and 22, to 22 μ for culture 1. The data (Table 3) show a similar difference in the mean diameters of the urediospores of various cultures. Culture 14 had the smallest mean diameter of urediospores, 17.18 μ. Culture 22 is scarcely larger, with a mean diameter of 17.21 μ. There is no correlation here of small urediospore size and aecial host, for 14 goes to Impatiens and 22 to *Clematis virginiana*. Two other cultures (18 and 21), having Impatiens for aecial hosts, have fairly large urediospores, with mean diameters of 19.84 μ and 18.94 μ respectively. The culture having the largest urediospores is number 1 (*Thalictrum* spp. aecial host), with a mean diameter of 21.07 μ.

The teliospores of these 8 cultures also showed differences. In table 4 it will be noted that, for the species as a whole, the teliospores range in diameter from 8 to 21 μ, the extremes being poorly represented. Two cultures are outstanding for narrow teliospores, culture 18 with a mean diameter of 10.77 μ, and culture 21 with a mean diameter of 10.67 μ. The teliospores with the largest mean diameter were obtained in culture 11, which was outstanding for scant and tardy production of teliospores.

The lengths of the teliospores of these cultures ranged from 24 to 53 μ (Table 5). Culture 21 is outstanding for short teliospores, having a mean length of 32.80 μ. Culture 1 was the largest, with a mean of 39.42 μ.

These data show that there is not necessarily a correlation between relative sizes of urediospores and teliospores. For instance, culture 11 has the widest teliospores of the 8 cultures, but the mean diameter of its urediospores is comparatively quite small. Teliospores of culture 18 have a comparatively small mean diameter, but the urediospores of this same culture are fairly large. However, culture 1 has both the largest urediospores and the largest teliospores of the 8 cultures.

Morphologically, a few of the cultures showed some differences. Both 22 and 14 could be separated from the remainder of the cultures because of their small urediospores (Fig. 1), but not from each other. Culture 21 is separable from the others because it has the smallest teliospores (Fig. 2). Culture 18 is separable from the rest by its teliospores of distinctive shape (Fig. 2), which are mostly narrowly wedge-shape and, therefore, differ from the teliospores of the other cultures.

Cultural Experiments with Puccinia tomipara

Culture 20. On Bromus ciliatus and Thalictrum dioicum. Culture 20 originated from aecia collected on *Thalictrum dioicum* near Ann Arbor,

TABLE 5.—*Frequencies of the lengths of the teliospores of 8 cultures of Puccinia rubigo-vera*[a]

Culture	Classes according to spore length															Mean length
	24–25 μ	26–27 μ	28–29 μ	30–31 μ	32–33 μ	34–35 μ	36–37 μ	38–39 μ	40–41 μ	42–43 μ	44–45 μ	46–47 μ	48–49 μ	50–51 μ	52–53 μ	μ
1		2	2	5	14	24	35	18	16	15	4	8	4	2	1	39.42
8			3	9	16	17	40	18	17	17	5	1	5	2		38.75
11			5	12	14	26	44	15	22	9	2	0	1			37.03
13	2	6	7	13	18	22	39	11	14	4	4	7	3			35.96
22		1	2	10	14	11	32	16	12	20	11	8	6	4	3	39.10
14			6	13	14	30	33	22	14	6	6	4	1	1		37.85
18	2	6	9	14	15	11	27	27	18	14	4	3				36.85
21	2	9	15	26	35	28	19	11	1	4						32.80

[a] One hundred and fifty spores of each culture were measured.

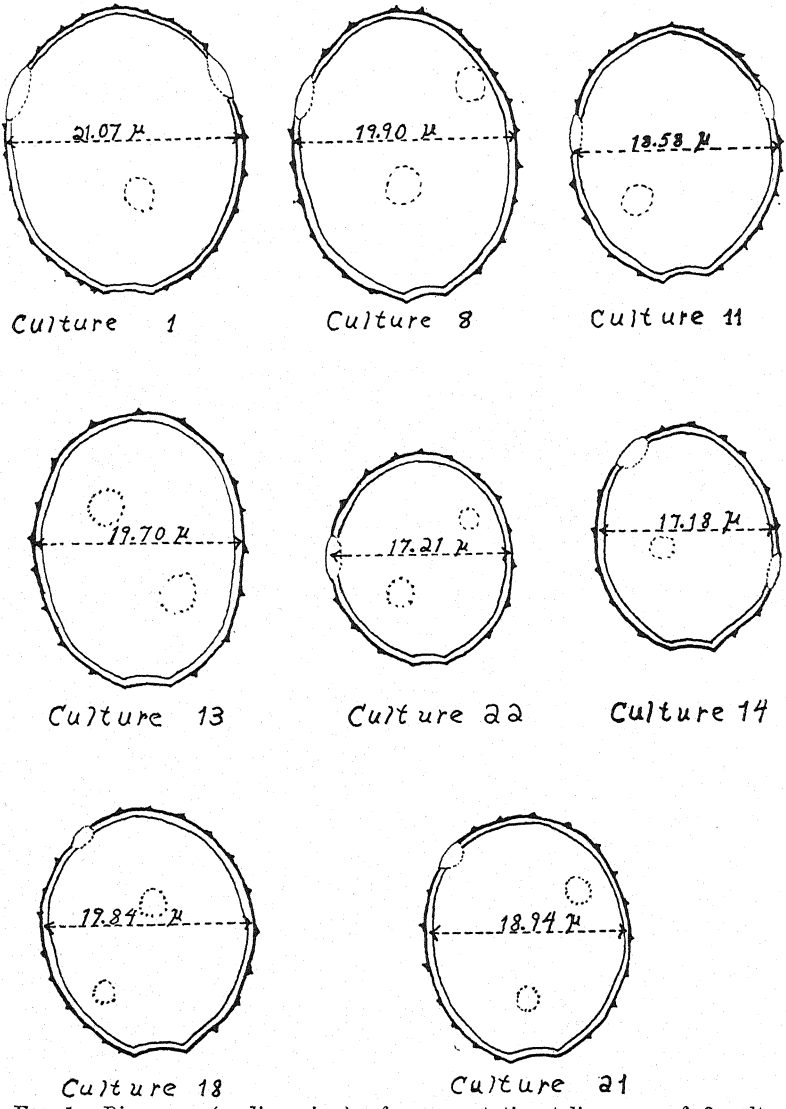


FIG. 1. Diagrams (median view) of representative teliospores of 8 cultures of *Puccinia rubigo-vera* to show differences in the mean dimensions of the spores of each culture.

May 30, 1932. In close association with these aecia were the weathered remains of a species of Bromus, identified the following autumn as *B. ciliatus*. On the over-wintered leaves and sheaths from the previous season numerous telia of the leaf rust type were found. The teliospores were found to be multicellular and the rust was therefore, *Puccinia tomipara*.

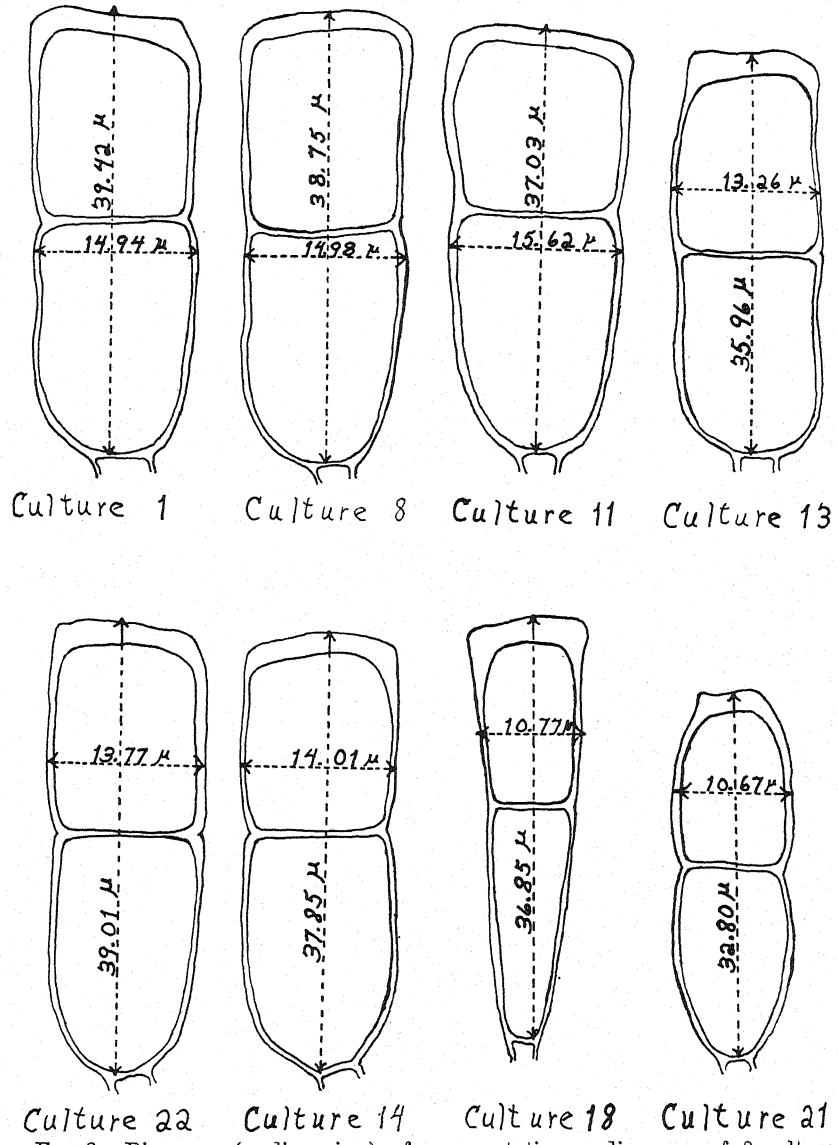


FIG. 2. Diagrams (median view) of representative urediospores of 8 cultures of *Puccinia rubigo-vera* to show differences in the mean diameters of the spores of each culture.

The aecia were used to inoculate several species of Bromus in an attempt to find a favorable grass host. Uredia appeared on *B. purgans* only. This uredial culture was multiplied from time to time throughout the summer. Early in July telia began to appear; these continued to appear

in gradually increasing amounts, so that by the end of November only telia were produced. Examination showed that about 95 per cent of the spores of each telium were multicellular and many were muriform. The telia thus produced in the greenhouse from the original aecial collection, were overwintered in the usual manner. They were germinative even as early as March 8, 1933. A potted plant of *Thalictrum dasycarpum* was inoculated on March 9, resulting in pycnia on March 21 and open aecia on March 31. Seedlings of *B. ciliatus* (1649) and *B. purgans* (1289) were inoculated from these aecia, resulting in a heavy infection on both grasses. The culture was propagated in the usual manner. On May 17, the first telia were collected. Ninety to 95 per cent of the spores of these telia were multicellular, many of them consisting of 6 to 8 cells (Fig. 3). The infection of *T. dioicum* and

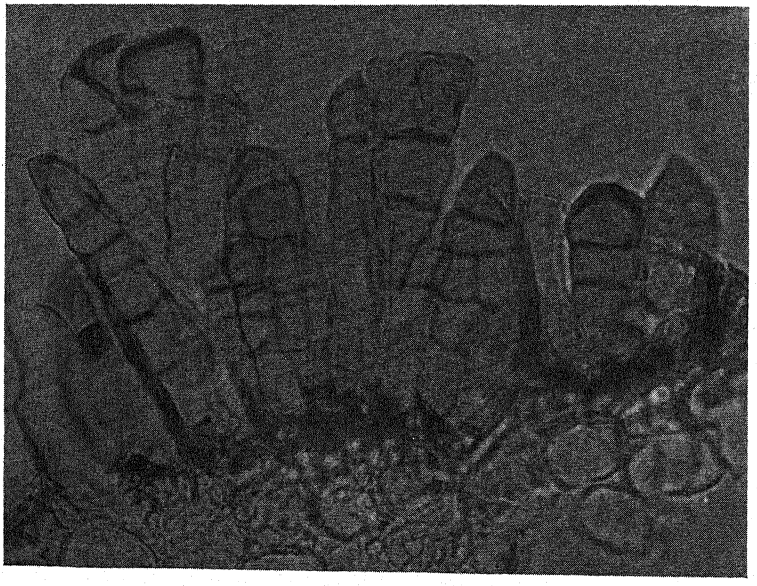


FIG. 3. Vertical section through a telium of *Puccinia tomipara* showing the multicellular structure of the teliospores. × 600. (Photograph by E. B. Mains.)

T. dasycarpum was repeated several times. Grass inoculations with aecia collected in the field on *T. dioicum* and *T. dasycarpum* have shown that aecia of *Puccinia tomipara* and *P. rubigo-vera* (see culture 13) may occur side by side on leaves of the same plant and, no doubt, on the same leaf.

Other species of Thalictrum were tested for susceptibility to *Puccinia tomipara.* Inoculations of *T. fendleri, T. flavum,* and *T. glaucum* resulted in the production of pycnia and aecia on each.

The host range of *Puccinia tomipara* within the genus Bromus was studied in connection with a similar study of the Bromus culture (Culture

23) of *P. rubigo-vera.* The writer has observed both species present on the same grass hosts in the field, sometimes even intermixing on these grasses, as indicated above (Culture 23). *Puccinia tomipara* has been found in the field on *Bromus ciliatus* and *B. altissimus.*

Inoculations of a series of *Bromus* species with *Puccinia tomipara* have given the following results:

DISTINCTLY SUSCEPTIBLE: *Bromus altissimus* (1795), *B. ciliatus* (1649), *B. purgans* (1289).

MODERATELY SUSCEPTIBLE: *Bromus rigidus* (1384, 1452), *B. rubens* (709, 1502).

DISTINCTLY RESISTANT: *Bromus adoensis* (1441, 1331, 815), *B. arduensis* (1332), *B. arvensis* (708, 1297), *B. brizaeformis* (1442), *B. commutatus* (716, 771, 1349, 1449, 1298), *B. hordeaceus* (719, 1508), *B. intermedius* Gus. (1285), *B. japonicus* (1347, 1551), *B. kalmii* (1541), *B. lanuginosus* (1550), *B. macrostachys* (797, 714, 705, 1446), *B. madritensis* (713, 1362, 1385, 1504, 1447), *B. maximus* (1427), *B. racemosus* L. (711), *B. rigidus* (1481, 1501, 698, 1505), *B. rigidus gussonii* (1506, 1333), *B. secalinus* L. (707), *B. sterilis* (1503), *B. squarrosus* (1450), *B. tectorum* (1547, 768, 1545, 712), *B. uniloides* (1507, 1451, 718, 750).

The specialization of this rust to such a few species of Bromus is interesting. Of 26 species of Bromus inoculated, only 3 closely related species are favorable hosts. Another fact to be noted is the lack of marked reactional differences between different collections within grass species. Only one species (*B. rigidus*) evinced any such differences.

DISCUSSION

It is shown that the multicellular character of the teliospores of *Puccinia tomipara* is constant. No reduction in the percentage of multicellular spores produced was observed while the rust was carried in culture from aecia to telia, back to aecia and then to telia again. Approximately 95 per cent of the teliospores of the telia ultimately produced from the second generation of aecia were multicellular. It is concluded, therefore, that this rust deserves specific rank.

Nine cultures of *Puccinia rubigo-vera* on wild grasses have been shown, in the experimental data presented, to be physiologically distinct. The distinctions have been determined on the basis of the infection type of 8 of the cultures on seedlings of 46 collections of wild grasses of the genera Agropyron, Elymus, Hordeum, and Hystrix, and of one (Culture 23) on species of Bromus. The specialization of the cultures to their aecial hosts serves further to distinguish some of the cultures. Two cultures (22 and 23) produce aecia on *Clematis virginiana;* three (14, 18, and 21) on *Impatiens biflora,* one (1) on exotic species of Thalictrum and one (13) on two native

species of Thalictrum; the aecial hosts for two (8 and 11) were not determined.

According to the concept employed by some investigators (Eriksson (8), Klebahn (17), Arthur and Fromme (4), Arthur (5), and others who have emphasized specialization to aecial host), a few of these cultures could be considered separate species. Culture 14, with aecia on *Impatiens biflora,* comes within the concept of *Dicaeoma impatientis* (Schw.) Arthur, (*Puccinia impatientis* (Schw.) Arth.) as given by Arthur and Fromme in North American Flora (4). The other two cultures (18 and 21), bearing aecia on Impatiens have urediospores somewhat larger than those described for that species. However, all three of these cultures having Impatiens for the aecial host conform well to the variety *P. rubigo-vera impatientis* (Arth.) Mains, as delimited by Arthur (5). Cultures 1 and 22, with aecia on Thalictrum and Clematis, respectively, agree with *D. clematidis* (DC.) Arth. as delimited by Arthur and Fromme (4) in North American Flora, except that the urediospores of culture 22 are too small. Culture 13, with aecia on Thalictrum, also might be placed in *D. Clematidis,* except in this case the teliospores are too small. This would indicate insufficient correlation between spore morphology of the leaf rust of the wild grasses and specialization to aecial hosts for a logical separation of species within the group, thus conforming to the conclusions of Mains (24), who recognized the whole group as an aggregate species, *P. rubigo-vera,* and erected 56 physiologic races, based on culture work of his own and that of previous investigators. Recently, Arthur (5) has recognized 6 varieties within the aggregate species, *P. rubigo-vera,* based chiefly on aecial- and telial-host restriction. Four of these, *viz., P. rubigo-vera tritici* (Eriks. and Henn.) Carl., *P. rubigo-vera secalis* (Eriks.) Carl., *P. rubigo-vera apocrypta* (Ellis and Tr.) Arth., and *P. rubigo-vera impatientis* (Arth.) Mains, are reasonably distinct because the aecia of each are restricted to a different family. In one case (the variety *impatientis*) this distinction is aided by the comparatively small size of the teliospores. The other two varieties, *P. rubigo-vera Agropyri*[4] Eriks.) Arth., and *P. rubigo-vera agropyrina* (Eriks.) Arth. have aecia on hosts of the same family (Ranunculaceae) and have essentially similar grass hosts. They are not morphologically distinct. It would be difficult to assign any of the 3 cultures of this study (nos. 1, 22, 13) having aecia on ranunculaceous hosts to one or the other of these two varieties.

Attempts to identify the 9 cultures of leaf rust of wild grasses with the 56 races of *Puccinia rubigo-vera* described by Mains (24) were unsuccess-

[4] Arthur (5) cites this as "*Puccinia rubigo-vera Agropyri* (Erikss.) n. comb. (*P. agropyri* E. and E)." Since this varietal name apparently applies to the *Puccinia Agropyri* of Ellis and Everhardt rather than to Eriksson's *P. dispersa Agropyri* (*P. agropyrina*) the variety, if maintained, should be designated as *Puccinia rubigo-vera Agropyri* (E. and E.) Arth.

ful. Culture 1, common in Michigan on quack grass, *Agropyron repens,* might be *P. rubigo-vera* sp. f. *persistens* (Plowright) Mains. It, however, differs sharply from that race in that infection was obtained (reaction type 3–4) on strains of *Hordeum jubatum, H. pusillum* and *A. tenerum.*

Culture 8 on *Agropyron repens* from Indiana is almost identical in its morphology with culture 1, and, like culture 1, is similar to the race *persistens.* Culture 8, however, differs from this race in its ability to infect strains of *Elymus canadensis.* If either culture 1 or 8 is to be interpreted as belonging to race *persistens,* then the host range of that race must be extended and these 2 cultures interpreted as physiologic forms, differentiated by strains of a few species of grasses.

Culture 11 has been definitely shown to produce a type 3–4 reaction on *Agropyron tenerum, A. dasystachyum, Elymus canadensis, E. glaucus, E. robustus, E. striatus* var. *arkansanus, E. virginicus, E. virginicus* var. *glabriflorus, E. virginicus* var. *submuticus, Hordeum murinum* (1548 only) and *Hystrix patula.* In this combination of hosts the culture approximates *P. rubigo-vera* sp. f. *elymicola* Mains, but on the basis of the results reported by Mains (24) the race *elymicola* does not infect *A. tenerum* or *Hordeum murinum* and does infect *H. gussoneanum.* In a similar manner each of the 9 cultures of *P. rubigo-vera* used in these studies could be shown to differ in grass-host range from any of the 56 races of Mains (24).

Culture 11 might also be considered to be race *septentrionalis* Mains, which Mains (24) based on the studies made by Fraser (11) of a race of *Puccinia clematidis* having aecia on *Actea rubra.* However, definite determination of the race identity of culture 11 can not be made, because Fraser (11) did not inoculate *Agropyron tenerum, A. dasystachyum, Elymus glaucus, E. robustus, E. striatus arkansanus* and *Hordeum murinum.*

The fact that none of the 9 cultures can be assigned to any of the races of *Puccinia rubigo-vera* distinguished by previous investigators is due in part to the fact that many of the grasses used by the writer were represented by 2 to several reactionally different collections. The situation in the case of culture 22 may be cited to illustrate this point. This culture bears its aecia on Clematis, and might be expected to conform to one of the 9 races connected with Clematis and recognized by Mains (24). On the basis of the reaction of the grasses he inoculated, culture 22 most closely approximates his race *indianensis,* but apparently cannot be included in this race because *Agropyron caninum* (1466 only), *A. tenerum* (1659) and *Elymus glaucus* (1539 only) proved highly susceptible (resistant to race *indianensis*). Thus it is seen that if the writer's series of grasses had not included those particular strains of these 3 species, culture 22 might well have been considered as *P. rubigo-vera* sp. f. *indianensis* Mains. It might

be considered a physiologic form of this race, distinguished by the reactions of differential strains of *A. caninum, A. tenerum,* and *E. glaucus.*

Likewise, the use of more than one collection of the grass species has rendered a few of the cultures separable from the others where such separation would otherwise have been impossible or unsatisfactory. Thus, cultures 1 and 8 would have been considered identical had the series of grasses not chanced to include *Elymus canadensis* (795, 1305, 1366, 1537), *E. glaucus* (1482), *E. virginicus* (1475), *Agropyron repens* (B.G. 13007) and *Hordeum gussoneanum* (1291). The other collections within these same grass species gave identical reactions to cultures 1 and 8. The situation in cultures 14 and 21 is similar, though perhaps less well-marked. These two cultures have the same aecial host (Impatiens) and would have been considered identical had not certain collections of grass species been included in the inoculations, *viz. E. canadensis* (843, 1305, 1366, 1537), *E. virginicus* (765), *A. caninum* (1466), *A. dasystachyum* (1656), *H. gussoneanum* (1421), *H. pusillum* (1650) and *Hystrix patula* (1477).

The manner in which the various strains within the wild grass species differ from each other is similar to the reactional differences that have been found in agronomic varieties of the cereals. The results of these investigations indicate that it would be possible for physiologic forms of the leaf rust of wild grasses to be distinguished one from the other by a standard set of strains or varieties within any one of several species of wild grasses.

The greater the number of strains or differentials used, the greater the possibilities of distinguishing divisions of the rust based on host specialization. If, as these studies indicate, there is in the races of leaf rust on wild grasses, a condition of physiologic specialization comparable to that in the races on the cereals (rye, wheat) almost innumerable forms might be differentiated if variously reacting strains of the wild-grass species are selected and employed.

In previous studies where physiologic forms have been distinguished by strains or agronomic varieties of a host species, only those belonging to one host species have been employed, as, for example, the use of agronomic varieties of wheat to distinguish physiologic forms of *Puccinia rubigo-vera tritici.* Apparently, no attempt has been made to determine the reaction of such physiologic forms on strains or varieties of other species of hosts. It is obvious that such a procedure would soon become very involved and hardly feasible. On the other hand, it is evident that if divisions of species of rusts are made on the basis of specialization to several species of hosts, intraspecific reactional differences should be taken into consideration in order that the results of various investigators may be comparable. As in the study of physiologic forms of the cereal rusts, a standard selected series of strains of the host species involved could be employed.

From the above discussion, the conclusion may be drawn that physiologic specialization in the leaf rust of wild grasses presents a situation quite comparable to that of the leaf rust of cereals and that interpretations of culture work should involve a consideration of the intraspecific reactional differences between collections of wild-grass species.

SUMMARY

Forty-six collections representing 18 species and varieties of wild grasses of the genera Agropyron, Elymus, Hordeum, and Hystrix, were studied with regard to their reaction to 8 cultures of *Puccinia rubigo-vera.*

The reactions of the wild-grass collections to these 8 cultures of leaf rust showed that the cultures are physiologically distinct.

Twenty-six species of Bromus were inoculated with 2 rusts of brome grass, 1 of which was a culture of *Puccinia rubigo-vera,* and the other, *Puccinia tomipara.*

Marked intraspecific reactional differences were noted for various collections of wild-grass species when inoculated with the various cultures of leaf rust.

In view of the fact that strains within wild-grass species may differ markedly from one another with regard to reaction to *Puccinia rubigo-vera,* it becomes evident that if the results of studies of host specialization of this rust are to be comparable, grasses of known genetic constitution should be employed.

Puccinia rubigo-vera on wild grasses presents a degree of physiologic specialization quite comparable to that characteristic of the races of cereal rusts.

Certain cultures of *Puccinia rubigo-vera* differed rather markedly from the others in mean dimensions of the spores. Cultures having the smallest urediospores do not necessarily have the smallest teliospores. Attempts to correlate spore size with host specialization were unsuccessful.

The multicellular character of the teliospores of *Puccinia tomipara* remained constant, through two generations. It is concluded, therefore, that this rust deserves specific rank.

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SEROLOGICAL EVIDENCE IN PLANT-VIRUS CLASSIFICATION

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The discovery that the injection of rabbits with plant-virus extracts induces in such animals the production of antibodies exhibiting various actions on the virus extracts has led to attempts to utilize these reactions in attacking a number of plant-virus problems. Matsumoto and Somazawa (14, 15) have shown that it is possible to follow the invasion of tobacco tissues by tobacco-mosaic virus by means of the precipitin reaction. Recently, Beale (3, 4) has attempted the use of the precipitin test in determining the virus concentrations of tobacco-mosaic extracts. It has been suggested by Birkeland (5) and Beale (3, 4) that the serological reactions might be of use in the identification and classification of plant viruses. The present paper is designed to present a study of this phase of virus serology.

HISTORICAL

Precipitin reactions have been described for tobacco-mosaic extracts by Beale (1, 2) and others and for some constituent or constituents of the potato virus group by Dvorak and others (10). Gratia (11), who first tested 2 different plant-virus sera in the same experiment, showed that tobacco mosaic and a potato virus are not cross-reactive. Beale tested tobacco-mosaic serum against extracts of a number of other plant viruses with negative results, and this finding is suggestive that tobacco-mosaic virus is serologically distinct from these other viruses (1, 2). However, she was unable to perform the reciprocal tests showing that each of these heterologous virus materials was precipitinogenic against its proper serum. Because of the possibility that a negative reaction may be due to a lack of suitable antigen rather than to a lack of close relationship, it is commonly considered necessary in plant serology that all reactions be reciprocally tested before they may be considered as significant in relationship studies (17).

In 1934, Birkeland (5) reported precipitin results with a number of plant-virus antisera, namely sera for tobacco-mosaic, cucumber-mosaic, and "spot-necrosis" viruses. Each virus extract was precipitated by its homologous antiserum, but not by the other 2 heterologous sera. Precipitating sera for tobacco ring spot were not obtained. Birkeland's "ring spot" virus, obtained from Johnson (Johnson's tobacco virus V), is apparently the same

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virus as has been used in the present study and designated as "potato ring spot." This virus has been found by the writer to be serologically very closely related to the latent-mosaic virus of potato (8), and since the "spot necrosis" (Johnson's tobacco virus IV) and the "attenuated spot necrosis" used by Birkeland each reacted uniformly with his "ring spot," one may conclude that all these viruses belong in the latent-potato-mosaic serological group or contain elements of this group. Birkeland's "ring spot," obtained from Henderson, is Wingard's tobacco ring spot, caused by an entirely distinct virus, and the one referred to by the writer in this and other papers as tobacco ring spot.

The writer has shown the antigenic independence of tobacco-mosaic, tobacco-ring-spot, and cucumber-mosaic viruses by means of the specific neutralization reaction. In addition, this reaction has brought evidence of the close relationship of certain symptomatologically distinct strains of each of these viruses (7). In a paper presented at the 1934 meetings of the American Association for the Advancement of Science (8), the writer reported precipitin and complement-fixation reactions indicating a close relationship between a score of strains of tobacco-mosaic virus and between several strains of the latent-potato-mosaic virus, namely the ordinary latent or X-virus, British Queen streak virus, potato-mottle virus, and potato-ring-spot virus. The same paper also indicated the antigenic independence of the viruses of tobacco mosaic, latent mosaic of potato, mild mosaic of potato, aucuba mosaic of potato, cucumber mosaic, and tobacco ring spot. The vein-banding virus of potato was found to react so strongly with cucumber-mosaic virus as to indicate that it is a strain of the latter, and the reactions also suggested that severe etch of tomato was distantly related to tobacco-mosaic virus.

MATERIALS AND METHODS

It is relatively easy to obtain potent antisera to tobacco-mosaic virus and the latent-mosaic virus of potato, but using the techniques heretofore described for preparing sera for these viruses, complete failure was experienced with all of the other viruses mentioned in the present study. The titers obtained, as will be seen below, are not very high, relatively speaking, and other measures must be taken to insure potent immunization.

Virus Stocks

The 20 tobacco-mosaic virus strains used in the present study had been repeatedly purified by the single-lesion method. The strains studied were selected from a larger number of available strains, being chosen because of their striking distinctness from one another and from tobacco mosaic. The sources of the different virus materials were as follows:

Virus	*Source*
Tobacco mosaic and its yellow, white, and slow-moving strains	J. H. Jensen
Masked tobacco mosaic	F. O. Holmes
Latent potato mosaic	E. S. Schultz
Potato ring spot	J. Johnson
British Queen streak	E. S. Schultz
Potato mottle	E. S. Schultz
Mild mosaic of potato	E. S. Schultz
Aucuba mosaic of potato	E. S. Schultz
Vein banding of potato	E. S. Schultz
Severe etch of tomato	W. D. Valleau
Tobacco virus 10729	W. D. Valleau
Cucumber mosaic	R. H. Porter
Tobacco ring spot	S. A. Wingard
Two mechanically transmissible viruses of pea, tentatively referred to as pea-mosaic viruses No. 2 and No. 3	H. T. Osborn

Each of these viruses was tested from time to time for purity, as far as this could be established by inoculations on differential hosts. In the course of the work accidental contaminations were rare and were readily detected and eliminated.

Preparation of Immune Sera

Freshly expressed saps were found preferable to other virus preparations for immunization. With viruses as resistant to physical agencies as those of tobacco mosaic and the latent potato mosaic, expressed saps may be frozen, melted, and centrifuged before using, a procedure that serves to throw down considerable protein and render the extracts relatively clear and nontoxic, although highly infective and antigenic. With other viruses that are more susceptible to destruction on freezing, freshly-expressed centrifuged saps were used exclusively. With moderately resistant viruses, such as those of cucumber mosaic, tobacco ring spot, and aucuba mosaic of potato, intraperitoneal injections suffice, and 5–6 cc. of expressed sap injected in each of 8 inoculations, at intervals of 3–4 days, was the customary dosage. With the less stable viruses, such as severe etch, intraperitoneal injections of freshly-expressed sap proved unsatisfactory, and intravenous inoculations were used, the dosage usually being 2–3 cc. of centrifuged, freshly expressed sap given in each of 8–16 inoculations at 3–4 day intervals. Frequently a combination of these methods (*e.g.*, 4 cc. intraperitoneal + 2 cc. intravenous, per inoculation) proved more satisfactory with such viruses.

The writer has experienced very little loss of animals inoculated intraperitoneally (3 of 67 animals, inoculated intraperitoneally, died during the

period of immunization), but loss from the venous-inoculation method was more considerable (11 of 45 animals inoculated intravenously died during immunization). The animals almost invariably showed a regular and important gain in weight during immunization, this gain averaging 300–500 gm. per month. Accidental death was usually due to violence of reaction to the alkaloids of the inoculated saps, or less frequently to septicaemia or embolism. Rabbit loss has been generally attributed to anaphylaxis in the plant serological literature, but this is not believed to be the usual cause of loss in ordinary work. Various species of Nicotiana, *Solanum tuberosum* L., *Lycopersicon esculentum* Mill., and *Vicia faba* L. were the vehicles of immunization antigens. Of these, the potato proved to be most highly toxic, and satisfactory sera from antigens in this host were best obtained after dialyzing the expressed saps prior to immunizing, a process that removed the toxins without affecting the virus antigenicity. Immediately after venous inoculation with tobacco saps, rabbits usually experience a severe reaction of short duration, marked by spasmodic breathing and convulsions. This lasts but a few minutes, and within a half hour the animals look and act normally.

With viruses that do not immunize readily, a blood probe usually was made from each animal after the 8th and 12th or later inoculations, in order to determine whether or not the animal was sufficiently immune. A sample of 15 cc. of blood was taken 6 days after the last inoculation and following 24 hours of starvation. If the blood reacted unsatisfactorily, inoculations were resumed the following day; if satisfactory, the animal was exsanguinated from the carotid artery 8–10 days after the last inoculation. The blood was allowed to clot for an hour or two at room temperature, centrifuged twice, and the serum was at once frozen until needed for serological testing.

Precipitin Testing

All sera used for precipitin testing were first absorbed by adding 2 parts of expressed sap of the healthy host used in inoculation, and incubating for 2 hours at 37° C. and then overnight in the cold. They were then centrifuged clear and used in testing. Occasionally 3–5 parts of healthy juice were necessary, *e.g.*, with potato, but ordinarily 2 parts were sufficient to eliminate completely any reactions against the proteins normally present in the virus host. It was found necessary for the healthy juice to be in the same condition of preservation as the virus extracts later to be tested.

Precipitin tests were performed in tubes approximately 50×5 mm. (inner diameter), 0.2 cc. each of absorbed serum and virus dilution being pipetted into the tubes and mixed. The pipettes were not tested for inaccuracies in graduation, as the quantitative features of the precipitin reaction are hardly accurate enough to make such a measure of practical

importance. After mixing, a procedure facilitated by the use of a small mechanical shaker, the tubes were incubated at 37° C. for 2 hours and then read; then the precipitate was allowed to settle overnight in the refrigerator. The following morning further readings were made. Only settled flocculations were considered significant and then only when normal serum controls and healthy host-material controls were negative. A single precipitin experiment customarily involved from 100 to 600 tubes.

It is possible to determine precipitin titers either by progressively diluting the serum and testing each dilution against a constant virus extract, or by progressively diluting the extract and testing each dilution against a constant serum dilution. The latter has been found far preferable, for the following reasons: The *occurrence* of a precipitin reaction is independent of the method used, and, within limits, of the non-specific substances incidentally present in serum and sap. Given a positive reaction, however, its *strength* (*i.e.*, the amount of precipitate formed or the dilution to which this can be recognized) is conditioned by the serologically inert proteins also present in the juices. Serum contributes the greater part of all inert protein present in a serum-virus mixture. Hence the progressive dilution of the serum constantly reduces not only the active antibody but the inert substances that give to the virus reaction its volume. Thus progressively diluting the serum, the reaction observed falls short of the titer determined by virus dilution with constant serum, and introduces an added variable. The amount of protein present in plant saps is so inconsiderable, on the other hand, that its dilution, in the presence of undiluted serum, fails appreciably to distort the reaction observed. Therefore, in the present study it has been the practice to hold the serum constant (1:3 to 1:6 of absorbant),[2] and progressively dilute the extracts in a series, 1:1, 1:2, 1:4 . . . 1:512. All dilutions were made in 0.85 per cent NaCl.

The ring test also has been used. The ring test has proved less satisfactory than the flocculation test, however, because of the impossibility of a uniform layering and because frequently the ring titers were lower than those by flocculation.

Test antigens were customarily prepared in the following manner: Expressed saps of healthy and diseased plants were centrifuged, and to 20 parts of each sap was added 1 part of 17 per cent NaCl solution. The saps after shaking were frozen for 24–48 hours, melted, centrifuged, strained through cheesecloth, diluted in series with 0.85 per cent NaCl solution, and tested. Such solutions, if usable, were free from green coloring material and clear. Healthy host controls were prepared in exactly the same manner as virus extracts. It was found to be desirable wherever possible to

[2] As is customary in serological literature, 1:3 is read "one in three," signifying a concentration of 33%.

test the infectivities of test extracts at the time of precipitin testing. This was regularly done in the present study.

Complement Fixation

Beale reported positive complement-fixation tests with tobacco-mosaic materials (1, 2), but her results were not wholly satisfactory because the anti-tobacco-mosaic sera fixed complement so strongly in the presence of healthy-tobacco extract that the only positive observations were of quantitative differences. The following technique gave excellent qualitative results with tobacco-mosaic virus and the latent-potato-mosaic virus, eliminating normal host reactions completely. The sera were prepared from virus materials propagated in tobacco. The test antigens consisted of the same and heterologous viruses propagated in other hosts, *viz.*, pepper, tomato, beet, phlox, and zinnia. No cross-reaction whatever was observed with the healthy host material, and positive reactions occurred with appropriate virus materials in dilutions of 1:1000 or higher. The details of the system used were as follows:

.3 cc. inactive virus-immune serum (1:20) + 1 cc. virus extract (1:1, 1:3, 1:10 . . . 1:1000) + 2 units complement (.2 cc. of 1:10 to 1:20 fresh guinea-pig serum) + 1 hour at 37° C. + .5 cc. sensitized red cells of sheep (= .25 cc. 4 per cent washed sheep cells + .25 cc. inactive anti-sheep amboceptor at 1:1500 + 1 hour at 37° C.) + 1 hour at 37° C. The customary controls were employed for determining the possible hemolytic or anti-complementary actions of virus-immune serum (often hemolytic below 1:20), virus extracts (usually hemolytic or anti-complementary at 1:1, 1:3, and sometimes 1:10, and with tomato at 1:30), and of the other constituents of the reaction. The reactions obtained were very striking, the end points sharp, and the titers high. A zone phenomenon was frequently observed, namely, the inhibition of specific reaction in the presence of excess virus extract, as well as in the absence of sufficient virus extract for maximum reaction.

Although highly satisfactory with tobacco-mosaic and the latent potato viruses, the complement-fixation reaction was negative with tobacco-ring-spot, cucumber mosaic, vein-banding, and potato-aucuba viruses. This is attributed to the fact that, in order for reaction to be error-free, the immune serum and the virus extract must each be diluted to about 1:20. This double dilution lies so far beyond the end point of precipitation with these viruses that even a complement-fixation reaction is not obtained.

"Conglutination" (Mez' Reaction)

Plant-virus materials also exhibit the reaction studied by Mez and termed by him "conglutination"[3] (16, 17). The reaction of Mez was

[3] The term "conglutination" has been used with 3 very different meanings in serology. "Conglutination" was first applied to the normal agglutination of blood corpuscles

utilized in the present study to a very limited extent. The technique, following as closely as possible the directions of Mez, was as follows: To 1-cc. samples of virus extract were added, respectively, .1, .05, .033, .016, .008, and .000 cc. of immune serum. Such mixtures were incubated 1 hour at 37° C. Then to each tube was added .45 cc. of fresh bovine serum ("conglutinin"). The tubes were again incubated at 37° C. and read after 20, 40, 60, 90, 120, and 150 minutes. The use of this technique was restricted to strains of tobacco mosaic.

EXPERIMENTAL

Making use of the techniques described above, the complement-fixation and precipitin techniques have been applied to some 40 strains and distinct types of plant viruses in approximately 10,000 individual tests. A conspectus of the results is given in tables 1 and 2. From these tables the following facts are evident:

1. Tobacco-mosaic virus, the latent-mosaic virus of potato, mild mosaic of potato, aucuba mosaic of potato, vein-banding of potato, tobacco ring spot, and Osborn's pea-mosaic virus No. 2 are all found to be distinct serological entities.

2. Tobacco-mosaic virus, as has been indicated by the studies of Jensen (12), gives rise to many strains, often varying widely in their symptoms. Serologically these strains show such close relationship that they are quantitatively indistinguishable by the precipitin and complement-fixation techniques. The 20 strains tested varied in symptoms on tobacco from a masked strain which caused no symptoms, through the ordinary green mottling field-type of tobacco mosaic, to strains that killed young plants in a short time. The list includes several forms that are locally necrotic on *Nicotiana sylvestris* Spegaz. and Comes, such as the aucuba mosaic of tobacco, although the majority are systemic in this host. It also includes Johnson's yellow tobacco virus VI and certain strains isolated by Jensen, which are characterized by an inordinate slowness in occupying the tissues of the tobacco plant.

3. Such a close serological relationship is shown between the vein-banding virus of potato and cucumber mosaic that one may well be justified in

by such substances as ricin, abrin, and crotin, which, at a relatively high concentration, cause a clumping of the corpuscles. (Miessner and Rewald. Die Konglutination der roten Blutkörperchen durch Ricinussamen. Ztschr. Immunitätsforsch. 2: 323–349. 1909). Later Bordet, Gay and Streng (Bordet and Gay, Ann. Inst. Pasteur 20, 467–498. 1906; Bordet and Streng, Centralbl. Bakt. I, 49: 260–276. 1909) described a reaction designated as "conglutination," in which a substance, "conglutinin," normally present in bovine serum, stimulates complement to function in allowing agglutination and often hemolysis of blood corpuscles by a weak agglutinin normally present in horse serum. The reaction designated by Mez as "conglutination" bears no relation to either of the 2 above, as may be seen from the description of the technique. It is better designated as "Mez' reaction."

TABLE 1.—*Synopsis of precipitin and complement-fixation tests with various plant virus types*

Virus extract:	Immune serum: Tobacco mosaic 9 strains	Tomato severe etch	Potato latent mosaic	Potato mild mosaic	Potato aucuba mosaic	Potato vein banding	Cucumber mosaic	Tobacco ring spot (green)	Tobacco ring spot (yellow)	Osborn's pea virus No. 2	Osborn's pea virus No. 3
Tobacco mosaic (20 strains)	1: 256 1: 1000 +	0 to 1: 32	0 0	0	0 0	0 0	0	0		0	0
Tomato severe etch	0 to 1: 4	1: 16	0		0	0		0	0		
Potato latent mosaic	0 0		1: 64 1: 1000 +		0 0	0 0	0	0		0	0
Potato mottle	0	0	1: 64 1: 480		0	0	0	0	0		
Potato ring spot	0		1: 64 1: 1000	0		0	0				
British Queen streak	0		1: 32 1: 60								
Potato rugose mosaic	0		1: 64			1: 16		0			
Potato mild mosaic	0		0	1: 16	0	0	0	0		0	
Potato aucuba mosaic	0	0	0	0	1: 16	0	0	0		0	
Potato vein banding	0	0	0	0	0	1: 32	1: 16	0		0	0
Cucumber mosaic Valleau's tobacco virus 10729	0	0	0	0	0	1: 16 1: 8	1: 16	0	0	0	0
Tobacco ring spot (green) Tobacco ring spot (yellow)	0	0	0	0	0	0	0	1: 16 1: 32	1: 16 1: 2 to 1: 16	0	0
Osborn's pea virus No. 2	0		0	0	0	0	0	0		1: 8	1: 8
Osborn's pea virus No. 3	0		0	0	0	0	0	0		1: 8	1: 8

Key: Representative precipitin titers not underlined, complement-fixation titers underlined.
Although the table is compiled from numerous experiments, the repetitions have been sufficiently extensive to render the results quantitatively significant to an approximate extent.

TABLE 2.—*Synopsis of precipitin and complement-fixation tests with various strains of tobacco mosaic*

Virus extract:	Immune serum: Tobacco mosaic	Tobacco aucuba	Masked tobacco mosaic	Jensen 101	Jensen 102	Jensen 104	Jensen 108	Jensen 302	Jensen 303
Tobacco mosaic	1: 1000 + / 1: 256	1: 64	1: 100 / 1: 16	1: 30 / 1: 512	1: 96	1: 128	1: 96	1: 30 / 1: 128	1: 128
Tobacco aucuba	1: 128	1: 64	1: 128	1: 256	1: 128	1: 64	1: 64	1: 64	1: 64
Johnson tobacco virus VI	1: 128	1: 64	1: 64	1: 128	1: 64	1: 64	1: 64	1: 64	1: 64
Masked tobacco mosaic	1: 30 / 1: 32	1: 32	1: 100 / 1: 4	1: 30 / 1: 64	1: 16	1: 16	1: 32	1: 30 / 1: 16	1: 64
Jensen tobacco mosaic 101	1: 100 / 1: 128	1: 32	1: 100 / 1: 16	1: 320 / 1: 40	1: 320 / 1: 96	1: 16	1: 320 / 1: 96	1: 320 / 1: 20	1: 320 / 1: 96
Jensen tobacco mosaic 102	1: 48	1: 128	1: 64	1: 320 / 1: 256	1: 320 / 1: 80	1: 64	1: 320 / 1: 48	1: 320 / 1: 20	1: 320 / 1: 48
Jensen tobacco mosaic 104	1: 96	1: 128	1: 64	1: 300 / 1: 128	1: 96	1: 64	1: 96	1: 128	1: 192
Jensen tobacco mosaic 108	1: 128	1: 64	1: 4	1: 64	1: 40 / 1: 16	1: 16	1: 40 / 1: 96	1: 64	1: 40 / 1: 192
Jensen tobacco mosaic 201	1: 256	1: 16	1: 16	1: 16	1: 192	1: 8	1: 96	1: 16	1: 96
Jensen tobacco mosaic 202	1: 192	1: 128	1: 128	1: 256	1: 96	1: 32	1: 96	1: 64	1: 96
Jensen tobacco mosaic 204	1: 32	1: 32	1: 32	1: 32	1: 128	1: 16	1: 32	1: 64	1: 64
Jensen tobacco mosaic 301	1: 64	1: 64	1: 32	1: 64	1: 128	1: 64	1: 64	1: 32	1: 128

TABLE 2.—(*Continued*)

Virus extract:	Immune serum: Tobacco mosaic	Tobacco aucuba	Masked tobacco mosaic	Jensen 101	Jensen 102	Jensen 104	Jensen 108	Jensen 302	Jensen 303
Jensen tobacco mosaic 302	1: 30 / 1: 192	1: 32	1: 100 / 1: 8	1: 30 / 1: 10	1: 40 / 1: 192	1: 16	1: 80 / 1: 96	1: 30 / 1: 10	1: 80 / 1: 96
Jensen tobacco mosaic 303	1: 384	1: 64	1: 32	1: 80 / 1: 20	1: 160 / 1: 192	1: 32	1: 320 / 1: 192	1: 80 / 1: 10	1: 320 / 1: 192
Jensen tobacco mosaic 304	1: 192	1: 16	1: 8	1: 16	1: 192	1: 8	1: 96	1: 8	1: 192
Jensen tobacco mosaic 305	1: 128	1: 128	1: 32	1: 128	1: 128	1: 64	1: 128	1: 64	1: 64
Jensen tobacco mosaic 306	1: 128	1: 128	1: 64	1: 128	1: 96	1: 32	1: 96	1: 128	1: 96
Jensen tobacco mosaic 309	1: 16	1: 16	1: 8	1: 16	1: 32	1: 8	1: 16	1: 16	1: 16
Jensen tobacco mosaic B-7	1: 16	The extracts enumerated to the left all contained appreciable amounts of infective material. Negative precipitin tests were obtained with extracts of the following, which were found to contain little or no infective material: Jensen Nos. 6, 103, 107, 110, 111, 112, 405.							
Jensen tobacco mosaic B-15	1: 32								

Key: Representative precipitin titers not underlined, complement-fixation titers underlined.
As the table was compiled from numerous experiments, the tests obtained are not quantitatively comparable, the significant feature being that all combinations are strongly positive.

regarding these as strains of the same virus type. This heretofore unrecognized relationship is further borne out by the fact that vein-banding and cucumber-mosaic viruses each produce on the cowpea, *Vigna sinensis* (L.) Endl., local brown necrotic lesions that are indistinguishable in appearance. Valleau's tobacco virus 10729 likewise reacted with both vein-banding and cucumber-mosaic sera.

4. Three sera were prepared for severe-etch virus. The first of these reacted positively with tobacco-mosaic virus, the second and third were negative with tobacco-mosaic virus. Tobacco-mosaic serum occasionally showed a weakly positive reaction with severe-etch virus. It is well known that with plant materials high-titered sera often react positively with extracts of distantly related plants, while lower-titered sera have a more restricted specificity. The first of the 3 severe-etch sera had a higher titer than the other 2. Thus a possible but distant relationship is indicated between severe-etch and tobacco-mosaic viruses according to the serological findings. This possible relationship awaits further confirmation from other types of investigation, particularly cross-immunization tests. If substantiated in other ways, the relationship is of particular interest, since the thermal inactivation points, one of the few seemingly solid bases of virus classification, are here very different.

5. The latent-mosaic virus of potato is representative of a group of closely related strains at present designated by such names as latent or healthy-potato virus, X-virus of potato, potato ring spot, potato mottle, British Queen streak, and spot necrosis. Böhme (6) has shown that a number of virus strains may be derived from the latent-mosaic virus of potato and from the potato-vein-banding virus. These viruses of a given type show little or no serological difference. They all cause systemic necrosis in pepper, a characteristic absent from all of the other viruses considered here, with the exception of potato aucuba mosaic, which causes a much more rapid and different-appearing necrosis of pepper. Accordingly, the serological tests thus far performed indicate that the potato viruses studied may be arranged in the following groupings:

a. *Latent potato-mosaic virus* (= X-virus of potato = healthy-potato virus), including as strains:
   - Potato mottle
   - Potato ring spot
   - British Queen streak
   - Spot necrosis (5)
   - Attenuated spot necrosis (5)

b. *Potato aucuba mosaic.*

c. *Potato mild mosaic.*

d. *Potato vein banding* (= Y-virus), including:

Cucumber mosaic
Tobacco virus 1027.9 (Valleau).

e. *Potato rugose mosaic,* as indicated by its serological reactions and as heretofore recognized, a mixture of a virus of the latent potato-mosaic group and potato-vein-banding virus.

6. Two legume viruses isolated by Osborn and designated here as pea-mosaic viruses No. 2 and No. 3, are serologically distinct from all of the other viruses mentioned above, but behave as 2 strains of the same virus type.

It will be noted that in most cases the reactions have been reciprocally tested. Fulfillment of this requirement is hampered at times by the fact that some of the low-titer sera, *e.g.,* mild-mosaic, cucumber-mosaic, and severe-etch sera, rapidly deteriorate in storage, and that it is these same sera that are most difficult to prepare. Indeed there seems to be some indication that the stability of a virus-immune serum varies directly as the stability of the virus concerned. The idea has been held by some serologists, notably Buchner, that antibodies are merely modified antigens. This view most adequately serves to explain their elaborate specificity. The indication that plant-virus antibodies partake of the degree of stability of their respective viruses is in accord with such a view.

The specificity of the serological reactions has been submitted to test in the identification of numerous unknown viruses. A few examples will suffice to illustrate this point. A single leaf of tobacco infected with a virus producing unusual symptoms was submitted for the purpose of determining whether or not it was tobacco-mosaic virus, as it was believed to have arisen from a tobacco-mosaic transplant. On testing, it was found to react not with tobacco-mosaic serum but with tobacco-ring-spot serum. Subsequent infection tests proved that the virus was actually that of tobacco ring spot. A sample of an unknown virus disease in a leaf of an unknown host was submitted with a sample of healthy host material. Precipitin tests showed within an hour that the virus was tobacco-mosaic virus, and this diagnosis was confirmed 2 days later by infection of *Nicotiana glutinosa.* A sample of potato mottle reacted with both tobacco-mosaic and potato-mottle virus sera. A contamination was suspected; the 2 components, tobacco-mosaic and mottle viruses, were isolated separately from the sample and purified, and thereafter the mottle sample showed only its proper reaction. A tomato-streak virus was submitted for identification. Precipitin tests showed it to be a variant of tobacco mosaic and this was confirmed by subsequent infection tests.

In a similar way, through the courtesy of colleagues, samples of the following viruses were submitted as unknowns and were in each case accurately identified as to group by means of the precipitin reaction: ordinary

tobacco mosaic, Porter's cucumber mosaic, tobacco ring spot, latent mosaic of potato, potato ring spot, potato vein banding, a strain of tobacco mosaic from petunia, and mixtures of tobacco mosaic plus cucumber mosaic and tobacco mosaic plus potato ring spot. Occasionally, in such tests of unknown samples, completely negative results have been obtained, but in no case tested has a virus been identified incorrectly.

Some of the serological reactions reported in tables 1 and 2 are illustrated in figures 1 and 2. Figure 1 illustrates the ring and flocculation

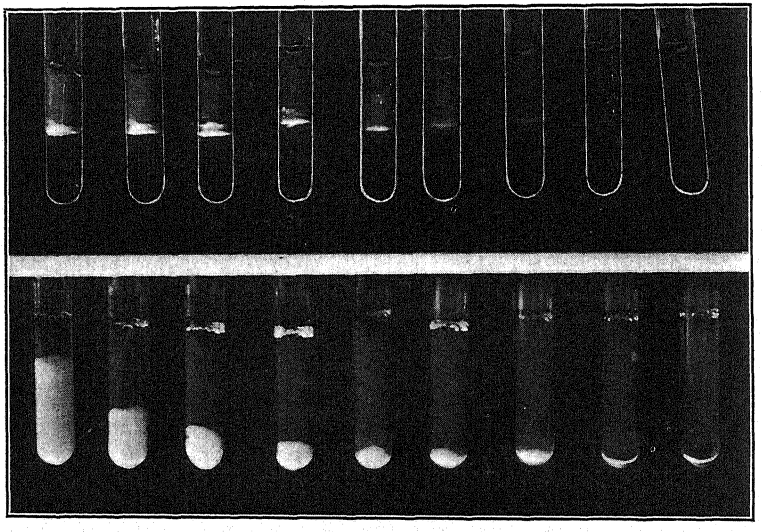


FIG. 1. Ring and flocculation precipitin tests between tobacco-mosaic virus antigen and tobacco-mosaic-immune serum. Explanation in the text.

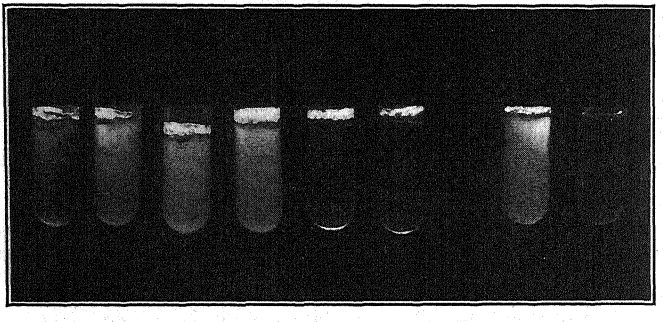


FIG. 2. Complement-fixation reaction between tobacco-mosaic virus antigen and tobacco-mosaic-immune serum. Explanation in the text.

tests applied to the same tobacco-mosaic materials. Each row of tubes from left to right illustrates the results of adding a constant amount of serum to

progressively weaker dilutions of tobacco-mosaic virus extract. The illustration of the ring test (above) shows the reaction as it appeared 1 hour after overlayering serum with virus; the tubes were then shaken, incubated, and the flocculation test is illustrated below as it appeared after further incubation.

A typical complement-fixation test is illustrated in figure 2. The 6 tubes to the left contain progressively-decreasing (left to right) concentrations of tobacco-mosaic antigen in the presence of a constant amount of immune serum and sensitized cells. The 7th tube is a control tube illustrating that the antigen alone (1:3) did not hemolyze nonsensitized cells, and the last tube is a control showing that the antigen alone (1:3) did not inhibit hemolysis. The other controls are not figured. Complete inhibition is seen in the 4th tube (antigen at 1:100), none in the 5th (1:300). The first tube (antigen at 1:3) shows only partial inhibition, a manifestation of the zone phenomenon in the presence of excess antigen.

Mez' reaction ("conglutination") was applied, according to the techniques previously described, to the interreactions of tobacco mosaic and its strains designated by Jensen as 201 and 302. At the same time, ordinary precipitin tests were performed with materials of a similar source. The results obtained showed that, in this case at least, Mez' reaction added nothing to the sensitiveness of the ordinary precipitin test. Difficulty also was experienced with turbidity of the controls. It is concluded that, with the viruses used in the experiments here reported, the precipitin technique is to be preferred, since it avoids the additional chance of error, when in Mez' technique another variable is added (beef serum) to an already complex system.

In conclusion it may be said that serological reactions offer a new criterion in the study of plant virus relationships, a criterion of particular interest and significance because of its subjective character. The writer has shown elsewhere (9) that there is strong evidence that the virus itself is the antigen involved in the reaction. Difficulty has been experienced in the past in deciding on which characteristic of many, stress in classification should be laid. Symptoms alone are insufficient as a criterion. The host ranges of apparently closely related viruses are not always identical. The importance of one criterion and the unimportance of another are largely matters of opinion. In serology we have an arbitrary technique in which opinion has little or no place. It is believed that the viruses are either proteid or that they are living entities, which, like other forms of life, would be basically proteid in constitution. It is probable that in either case a specific protein analysis thus would afford an insight into the basis of their natural classification. Imperfect though they may be, the serological techniques are, at the moment, our chief point of attack in the study

of the chemical basis of specificity (18). In this resides the value of the serological approach to the plant-virus problem, and its empirical results as introduced above support this thesis.

SUMMARY

1. A description is given of techniques that make it possible to prepare immune sera for a considerable number of strains and distinct types of plant viruses. In the present paper the precipitin and the complement-fixation techniques are particularly concerned.

2. The tests show the following virus types to represent distinct serological entities: tobacco mosaic, latent mosaic of potato, potato mild mosaic, potato aucuba mosaic, potato-vein-banding virus, tobacco ring spot, and Osborn's pea-mosaic virus No. 2.

3. From tobacco mosaic have been derived many strains of very close affinity serologically, although of very diverse symptomatology. The group of such uniformly interreactive viruses includes tobacco aucuba mosaic, Johnson's yellow tobacco virus VI, Holmes' symptomless tobacco mosaic, and many of Jensen's brilliant yellow, white, and slow-moving types.

4. The latent-mosaic virus of potato (= X-virus or healthy-potato virus) is representative of a group of serologically closely related virus strains embracing potato mottle, potato ring spot, and British Queen streak. According to Birkeland's tests (5), spot necrosis and attenuated spot necrosis also belong in the same group. Rugose-mosaic virus of potato reacts with both X-virus and vein-banding sera, confirming the present view that it is a mixture of these 2 virus types.

5. The vein-banding virus of potato and cucumber-mosaic virus are so strongly interreactive that these appear to be but strains of the same virus type. This relationship is supported by infection tests. Valleau's tobacco virus 10729 also shows marked affinities with these viruses.

6. A much more distant serological relationship between tobacco mosaic and severe etch of tomato is indicated by precipitin tests.

7. Two viruses isolated from legumes by Osborn and tentatively referred to as pea-mosaic viruses No. 2 and No. 3 behave serologically as strains of the same virus type, although both are serologically distinct from all of the other viruses studied.

8. The usefulness of seric reactions in plant-virus classification has been confirmed by tests of numerous virus samples submitted as unknowns. The results of such tests have shown a sustained accuracy in identification by means of the precipitin test.

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THE ANTIGENICITY OF THE PLANT VIRUSES

K. STARR CHESTER

(Accepted for publication April 15, 1935)[1]

Since the discovery that tobacco- and potato-mosaic juices precipitate in the presence of immune sera obtained from rabbits injected with these juices, while after absorption such sera show no reaction against the juices of healthy tobacco and potato plants, considerable emphasis has been laid on the question of whether the virus itself is the active antigen concerned or whether the antigen is an altered host protein, a virus derivative, or a complex of virus and host protein. Beale, Birkeland, and Matsumoto have all expressed the opinion that the active antigen is actually the virus; Beale, chiefly because tobacco-mosaic sera prepared from tobacco material react positively with extracts of several different plants in the Solanaceae, and with extracts of the closely related *Martynia louisiana* Mill. (1, 2) infected with tobacco-mosaic virus; Birkeland and Matsumoto, because their chemically-purified virus preparations showed specific serological activity with virus-immune sera (4, 8). In view of the importance of serological reactions in relation to studies on the nature of viruses and on virus classification, it has seemed desirable to devote some attention to the question of whether the viruses are or are not the active antigens. The present paper reports experiments bearing on this problem. The techniques employed and the virus materials used have been described in previous papers (5, 6).

EXPERIMENTAL

For purposes of orientation, the experiments of Beale and Birkeland mentioned above were repeated and supplemented, with results that may be summarized briefly as follows.

Tobacco-mosaic virus, purified by lead acetate precipitation, retained its antigenic capacity as indicated by the precipitin test. In samples of tobacco-mosaic virus from which more than 95 per cent of the non-virus constituents had been removed, the ratio, virus infectivity: precipitin titer, remained as in the unpurified controls. Purification of tobacco-mosaic virus with silver nitrate gave similar results. Tobacco-mosaic virus when purified by filtration through collodion filters of graded porosity in every case showed a retention of precipitinogenic power in the fraction, filtrate or residue, which retained the infective property, and a loss of both properties in the other fraction. Purification of different viruses by repeated freezing and thawing has likewise given analogous results. It was further found in other preliminary

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experiments that the strength of precipitin reaction varies directly as the amount of infective material in diluted samples of tobacco-mosaic virus, regardless of host species providing the antigenic juice.

PROGRESSIVE INACTIVATION OF PLANT VIRUSES BY VARIOUS MEANS AND RESULTANT EFFECT ON PRECIPITIN REACTIVITY

Certain of the plant viruses that have yielded precipitating sera (6) have thermal inactivation points widely removed from one another. Experiments were performed in order to determine whether or not the seric reactivity of heated virus extracts is lost at the temperature at which each virus is inactivated. The experiments were performed in the following manner.

A frozen stock of virus juice was cleared by centrifuging and distributed in 10-cc. centrifuge tubes in 7-cc. samples. A similar series of healthy host-juice samples was similarly prepared. Retaining unheated a tube of virus and one of healthy juice, tubes of each juice were heated for 10 minutes in a water bath at 5° C. temperature intervals over a range including the reported thermal inactivation point. After cooling the tubes, all were centrifuged and tested at once against absorbed immune and normal sera for the presence of precipitating activity. As soon as possible thereafter an aliquot of each sample was tested on plants suitable for measuring virus activity. The results of the infectivity tests and the precipitin reactions were then plotted on comparable scales, such as are shown in figure 1, which records the results of 4 representative experiments.

The precipitin titers, as given, indicate the highest dilutions to which the heated juices could be subjected and still retain a significant precipitin test (settled flocculation) on the addition of an equal volume of absorbed immune serum at dilution 1:3. The method of determining infectivity, as is indicated in the legend, was adapted to each virus in question.

It is seen from the figure that tobacco-mosaic juice subjected to 85° C. or 90° C. for 10 minutes showed little loss in infectivity, while in samples heated to 95° C. there was a sudden and virtually complete loss of infectivity. The precipitin reactions with the same samples of virus showed no loss in titer up to 85° C., a slight loss at 90° C., and complete loss at 95° C. Analogous results were obtained with the viruses of potato ring spot, tobacco ring spot, and potato vein banding, although in these cases the points at which infectivity and seric reactivity first disappeared were 70°, 65°, and 60° C., respectively.

In all cases there was a heavy protein coagulation at 65° C. This protein coagulation bore no relation to virus inactivation, as is shown by the fact that tobacco-mosaic virus was not inactivated at 70° C. in spite of this coagulation, while the vein-banding virus was completely inactivated at a temperature below the point of heavy coagulation.

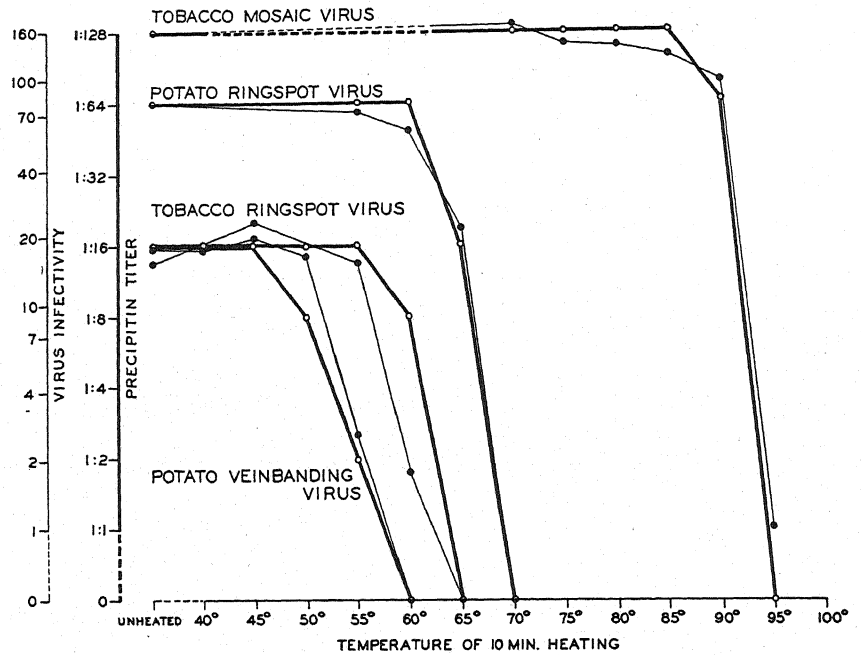


FIG. 1. The effect of 10 minutes' heating on precipitin reactions (coarse lines) and infectivity (fine lines) of certain plant viruses. Infectivity of tobacco-mosaic virus is given in terms of the number of local lesions produced per leaf on 16 leaves of *Nicotiana glutinosa;* that of potato-ring-spot virus in terms of the number of local lesions produced per 2 leaves using 100 leaves of *N. tabacum;* that of tobacco-ring-spot virus in terms of the number of local lesions per leaf on 30 leaves of *Vigna sinensis;* that of potato-vein-banding virus in terms of the number of cases of disease per 50 plants of *N. tabacum.*

All 4 of these viruses had been propagated in *Nicotiana tabacum* L. var. Turkish, and the fact that the seric inactivation curves and the virus inactivation curves nearly coincide for each virus, and that the curves are very different for the 4 viruses used, affords evidence of a dependence of the seric reactions on the presence of active virus, in spite of the common host matrix.

Experiments were also performed in a comparable way to test the effect of inactivation by pH change on the precipitin reaction. In this case, in different experiments, both lead-acetate-purified tobacco-mosaic juice and frozen unpurified tobacco-mosaic juice were used, and both gave essentially similar results. The method of procedure was to divide a virus stock supply into similar samples, to titrate each sample to a given hydrogen-ion concentration, using HCl or KOH, to hold a sample at that pH for a given length of time, usually an hour, and then to neutralize and centrifuge and perform the precipitin and infectivity tests at pH 7.0.

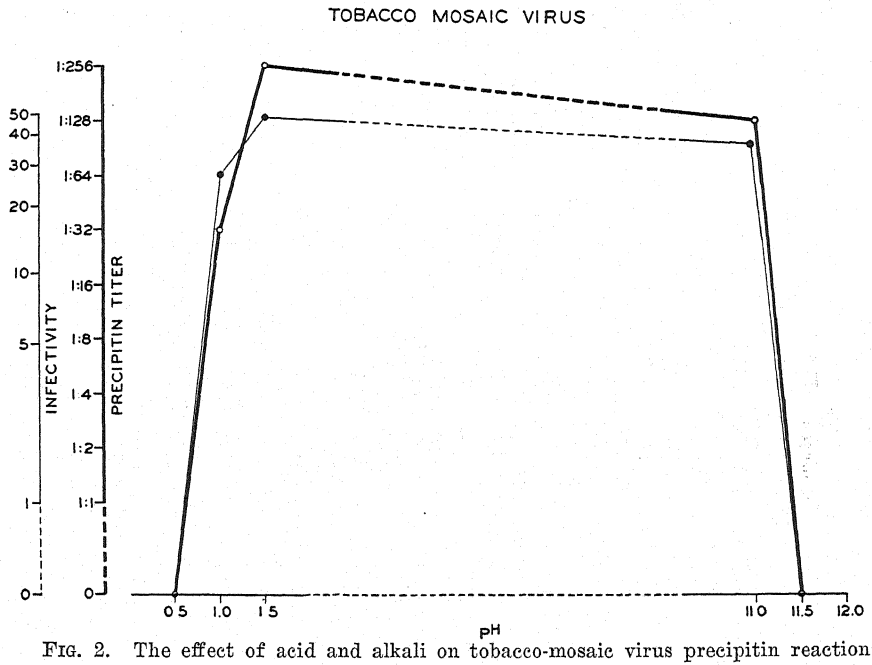


FIG. 2. The effect of acid and alkali on tobacco-mosaic virus precipitin reactions (coarse lines) and infectivity (fine lines). Virus infectivity expressed in terms of the number of local lesions produced per leaf on 16 leaves of *Nicotiana glutinosa*.

Figure 2 illustrates one of several such experiments, all of which gave analogous results. The experiment figured was performed with samples of titrated lead-acetate tobacco-mosaic material for which I am indebted to Dr. W. M. Stanley. From pH 1.5 to pH 11.0 there was no significant loss in either infectivity or precipitin reactivity. At pH 11.5 and pH 0.5 both reactions were completely lost, while at pH 1.0 there was a partial drop in both reactions. The experiment shows that with tobacco-mosaic virus inactivation by pH change the precipitin reaction is retained so long as virus infectivity is retained, and disappears at just the point at which infectivity is lost.

It was next thought advisable to focus attention on chemical virus inactivation, and accordingly a number of experiments were performed in which various viruses were progressively inactivated by chemical treatment and then tested for infectivity and seric reaction. The customary procedure was to divide a stock of frozen virus sap into 5-cc. samples and to add to each sample 5 cc. of reagent, this dilution of the chemical being considered in statements regarding the chemical concentration. After 1–2 hours of incubation at room temperature, each sample was dialyzed against tap water with continuous agitation and using cellophane membranes. After 4–24

hours of dialysis, when the samples no longer gave indication of the presence of the salt used, each sample was centrifuged and tested for precipitin reactivity and infectivity. Healthy host juice was similarly treated as controls. The results of 3 such experiments with tobacco-mosaic virus are plotted in figure 3.

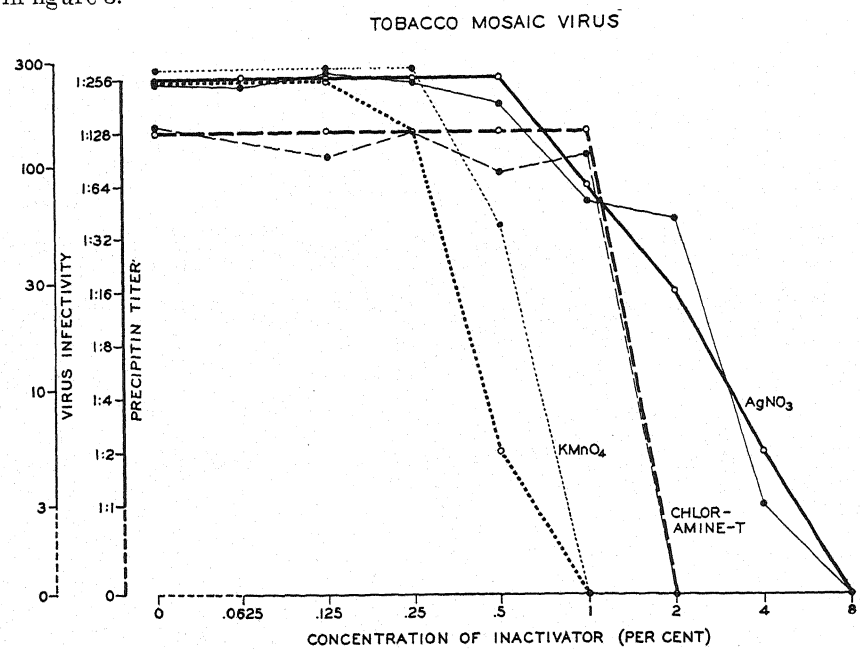


FIG. 3. The effect of certain chemical treatments of tobacco-mosaic virus on its precipitin reactions (coarse lines) and infectivity (fine lines). Infectivity given in terms of the number of local lesions per leaf produced on 16 leaves of *Nicotiana glutinosa* and *Phaseolus vulgaris*.

The figure shows that chloramine-T inactivates all virus at 2 per cent concentration, while practically none is affected at 1 per cent. The precipitin reaction is at its constant maximum with 1 per cent chloramine-T concentration, while at 2 per cent it entirely disappears. Similiarly, $KMnO_4$ eliminates both seric reactivity and infectivity completely at 1 per cent, both partially at .5 per cent, and fails to affect either appreciably at .25 per cent. $AgNO_3$ has a similar effect, the minimum inactivating concentration here being 8 per cent.

The tests with $AgNO_3$ were of special interest. $KMnO_4$ and chloramine-T are both oxidizing agents. $AgNO_3$, on the other hand, like salts of the heavy metals in general, is a powerful precipitant of proteins. In these experiments, in all cases, protein tests were made (trichloracetic acid) and the amount of precipitation produced by the chemical was noted. In the case

of silver nitrate a heavy precipitation of protein took place between salt concentrations .125 per cent and .5 per cent inclusive. The protein precipitated at 8 per cent, 16 per cent, and 32 per cent salt was no greater in apparent amount than at .5 per cent. In spite of this heavy precipitation at .5 per cent, the virus retained practically all of its infectivity and the precipitin test all of its strength. This fact in itself is strong evidence of the antigenic nature of the virus, since nearly all of the protein had been removed at a point at which the virus—notably resistant to ordinary protein precipitants —was neither precipitated nor inactivated, but retained in a pale yellow, water-clear solution.

A very striking phenomenon occurred in the tests with silver nitrate. In performing the precipitin tests with the crystal-clear, pale yellow extracts treated with virus at 0.5, 1, and 2 per cent, the serum precipitate, instead of being white, as in all other virus precipitin experiments heretofore performed, was a golden orange, light at 0.5 per cent, brilliant at 1 and 2 per cent. It is well known that the salts of silver customarily turn yellow. The solutions here tested contained very little protein but an abundance of virus and gave a copious specific yellow precipitin reaction. The yellow color could not have been due to any action of free silver on the serum, since all of the soluble silver that was unattached to protein (or virus) had been dialyzed out, as proved by chemical tests. Moreover, practically all of the non-virus protein had been removed by the silver nitrate precipitation. It also is known that the precipitate in the precipitin reaction contains the virus. It thus seems possible that the silver responsible for the yellow color of the precipitate is attached chemically to the virus forming a yellow soluble virus-silver combination that is infective and specifically precipitated by serum.

Representative experiments showing the effects of different concentrations of $KMnO_4$ and chloramine-T on the potato-ring-spot virus and the potato-vein-banding virus are illustrated in figure 4. In each case the precipitin reaction is retained at full strength as long as the virus infectivity is retained, and the two reactions are lost at the same concentration of chemical, both reactions diminishing at approximately the same rate.

The experiments with chemical treatments thus confirm those with heat, and indicate that the plant viruses studied lose their ability to react serologically at just the point at which they lose their infectivity.

SERIC REACTIONS IN RELATION TO HOST SPECIES SUPPLYING VIRUS SAPS

It has been shown in an earlier paper that each of a number of different plant viruses gives its specific virus seric reaction in spite of the common host matrix (6). The following experiments are representative of a number that were performed in an endeavor to supplement our knowledge of this relationship by testing the seric reactions of the same viruses in different hosts.

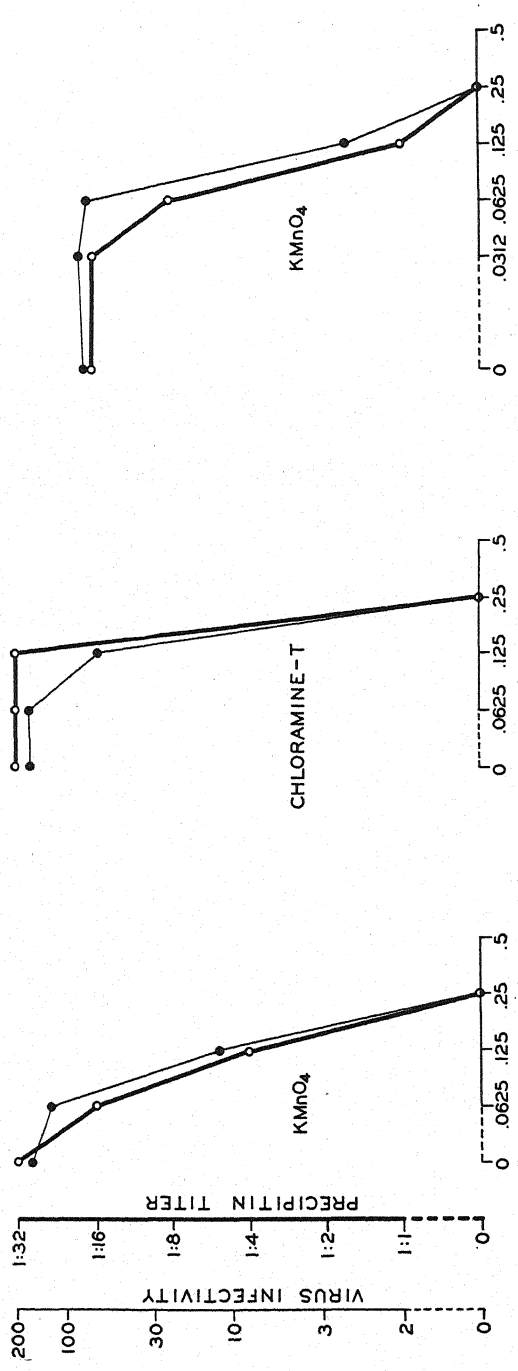


FIG. 4. Effect of certain chemical treatments of the viruses of potato ring spot and potato vein banding on serie reactions (coarse lines) and infectivity (fine lines) of the viruses. Infectivity of potato ring spot given in terms of the number of local lesions produced per leaf on 50 leaves of *Nicotiana tabacum*, of potato vein banding in terms of the number of cases of disease in 50 inoculated plants.

Tobacco-mosaic virus was propagated in tobacco, tomato, petunia, phlox, beet, and zinnia. The expressed saps of these plants were tested serologically against tobacco-mosaic serum prepared from tobacco sap and previously absorbed with healthy tobacco juice. The results are given in table 1.

It is seen from the table that the tobacco-mosaic virus reaction is positive in each case, while the sera are all negative against extracts of these healthy species. Moreover, the virus concentration varies, and is least in beet and zinnia, since the infection here is localized or only slowly systemic. The complement-fixation titers and the precipitin titers of the same extracts show an approximate proportionality to the amount of virus present as given by local-lesion counts. The experiment thus affords evidence that even in species as widely removed from one another as Compositae and Chenopodiaceae, separated in Gray's classification by 21 orders and more than 100 families, positive virus cross-reactions not only occur, but show no significant change in the ratio of precipitin reaction to virus concentration. In species so widely separated, common antigenic proteins do not normally occur.

Incidentally, the same table shows the greater absolute sensitivity of the complement-fixation reaction as compared with the precipitin reaction with the plant viruses. It also brings out the fact that the strengths of reaction by these two methods show similar relative changes from one test antigen to the next. Furthermore, the table illustrates the efficiency of the complement-fixation test by use of the expedient of testing a serum prepared from one host against virus extracts prepared in another host, a technique that eliminates the distorting nonspecific reactions heretofore obtained due to the strong fixation of serum with healthy host material of the same species as was used in serum preparation (1).

Table 2 illustrates an analogous experiment with strains of the potato latent-mosaic virus. The specific immune serum used had been prepared from the virus in tobacco and had been absorbed with healthy tobacco juice. All 4 strains of the latent-mosaic virus when propagated in tomato and the latent-mosaic virus when propagated in potato show strongly positive seric reactions with the absorbed serum prepared from tobacco. Similar experiments have shown that the latent-mosaic virus serum prepared from tobacco reacts specifically with the latent-mosaic virus in pepper, that potato-vein-banding virus serum prepared from tobacco reacts specifically with the vein-banding virus in pepper and potato, that cucumber-mosaic serum prepared from tobacco reacts specifically with cucumber-mosaic virus in zinnia, and that vein-banding and latent-mosaic virus sera prepared from potato react, alone or in mixtures, with their specific viruses in tobacco.

These experiments appear to establish the fact that there is no necessary relationship between host species and virus seric reaction, since, when numerous viruses are propagated in the same host, each gives its specific virus re-

TABLE 1.—*Precipitin and complement-fixation reactions with tobacco-mosaic virus in different hosts*[a]

Antigenic extract in saline dilution series	Concentration of virus[d]	Complement fixation titer[b]		Precipitin titer		
		Tobacco-mosaic serum	Three heterologous virus sera[c]	Tobacco-mosaic serum	Normal serum	Three heterologous virus sera[c]
Tobacco mosaic in tomato (Solanaceae)	150	1:1000+	0	1:240	0	0
" " " petunia "	100	1:1000+	...	1:100	0	...
" " " phlox (Polemoniaceae)	100	1:1000+	...	1:120	0	...
" " " beet (Chenopodiaceae)	10	1:300	...	1:30	0	...
" " " zinnia (Compositae)	30	1:480	...	1:30	0	...
Three heterologous viruses in tomato[c]	.....	0	...	.....	0	...
Healthy tomato	.....	0	0	.....	0	0
" petunia	.....	0	...	.....	0	...
" phlox	.....	0	...	.....	0	...
" beet	.....	0	...	.....	0	...
" zinnia	.....	0	...	.....	0	...
Physiological saline	.....	0	0	.....	0	0

[a] All sera prepared from virus material propagated in tobacco, and absorbed to remove precipitins for healthy tobacco proteins.

[b] All necessary controls employed and error-free, but not given in above table.

[c] Latent-mosaic virus of potato, potato-vein-banding virus, potato-aucuba-mosaic virus.

[d] Approximate number of local lesions per *Nicotiana glutinosa* leaf, using 1: 50 virus extract as inoculum.

TABLE 2.—*Precipitin and complement-fixation reactions with the latent-mosaic virus of potato and its strains*[a]

Antigenic extract in saline dilution series	Complement-fixation titer[b]		Precipitin titer		
	Latent-mosaic virus serum	Three heterologous virus sera[c]	Latent-mosaic virus serum	Normal serum	Three heterologous virus sera
Latent-mosaic virus of potato in tomato	1:1000+	0	1:60	0	0
" " " " " " potato	1:100	...	1:10	0	...
Potato ring spot in tomato	1:1000+	...	1:60	0	...
Potato mottle " "	1:480	...	1:60	0	...
British Queen streak in tomato	1:60	...	1:30	0	...
Three heterologous viruses in tomato	0	...	0	0	...
Healthy tomato	0	0	0	0	0
Physiological saline	0	0	0	0	0

[a] All sera prepared from virus material propagated in tobacco, and absorbed to remove precipitins for healthy tobacco proteins.

[b] All neccessary controls employed, and error-free, but not given in table.

[c] Tobacco-mosaic virus, potato-vein-banding virus, potato aucuba-mosaic virus.

action, while conversely, when the same virus is propagated in diverse hosts a common virus reaction is retained regardless of host.

DISCUSSION

The evidence thus far adduced regarding the antigenicity of the plant viruses may be briefly summarized as follows:

1. Tobacco-mosaic virus that has been subjected to purification by the use of lead acetate, acetone, trypsin, aluminum hydroxide, barium hydroxide (4, 8), alternate freezing and thawing, and $AgNO_3$ precipitation, retains its ability to react specifically with tobacco-mosaic immune serum prepared either from whole sap or from purified tobacco-mosaic juice. Moreover, the ratio, virus amount: precipitinogenic power, remains constant throughout such purification.

2. Viruses of tobacco mosaic (8), vein banding of potato, latent mosaic of potato, and tobacco ring spot, when subjected to progressive inactivating treatment by heat and by various chemicals, in every case lose their infectivity at just the points where their precipitin reactions are lost, and when approaching these points of complete inactivation their precipitating capacity diminishes at approximately the same rate at which they are being inactivated.

3. Immunization of rabbits with plant-virus extracts produces sera that specifically and quantitatively neutralize the viruses concerned in the presence or absence of precipitin reactions,[2] a result that is interpreted as due to the primary antigenicity of the viruses themselves (5).

4. The ease of immunization of animals and apparently also the stability of the sera (6) vary directly with the stabilities of the viruses used in immunization, as indicated by their resistances to heat and to aging.

5. Fractionating tobacco-mosaic extracts by filtration through graded membranes in each case shows the precipitating power to be retained in the fraction containing the infective material (4).

6. The precipitin and complement-fixation reactions are approximately proportional in strength to the amount of infective material present in diluted virus samples (3).

7. When numerous viruses are propagated in the same host species each gives its specific virus reaction (6), and when the same virus is propagated in species very widely separated taxonomically, the common serological reaction is retained, and, regardless of species, still varies in strength proportionately to the amount of infective material present.

It is concluded that the evidence now available is sufficiently strong to warrant the assumption that the antigens responsible for the plant-virus seric

[2] Chester, K. S. Independence of specific neutralization and precipitin reaction with certain plant viruses. (Unpubl. ms.). 1934.

reactions are the viruses themselves and not normal or derived constituents of diseased plants.

Matsumoto and Somazawa (7, 8) treated tobacco-mosaic extract with formalin and trypsin and found that the precipitin reaction was retained, although infectivity was partially or completely lost. Stanley, however, (9) has shown that inhibitory effect of trypsin, as of many other substances, is an effect on the host plant, not on the virus; and, accordingly, Matsumoto and Somazawa's trypsinized extracts very probably contained much or all of the tobacco-mosaic virus, while their infection tests were negative because of the effect of the reagent on the host plant. Thus this finding, which has been cited as unfavorable to the view that viruses are antigenic, is better interpreted on other grounds.

The fact that inactivated virus is not antigenic might seem to indicate that the viruses are fundamentally different from certain low forms of life. It is well known that dead bacteria retain their antigenic potency and specificity. It is by no means established, however, that the bacterial protoplast is the antigen in the bacterial reactions, and recent work suggests that the peripheral structures of bacteria are serologically important. It is to be expected that these superficial structures have a greater stability than the protoplast within, *i.e.*, that they retain their serological activity after the protoplast is killed. The findings with the plant viruses, on such a basis, offer no grounds for objection to a biotic conception of these agents.

SUMMARY

1. The findings of Beale and Birkeland, respectively, that within the Solanaceae the tobacco-mosaic precipitin reaction is independent of host species, and that purified tobacco-mosaic virus retains its specific precipitin reactivity, are confirmed. The latter finding is extended by the use of additional methods of purification.

2. When tobacco-mosaic, tobacco-ring-spot, potato-vein-banding, and potato-ring-spot viruses are inactivated by series of progressive strengths of chemicals, or by progressive degrees of heating, and when tobacco-mosaic virus is inactivated by progressive changes of pH, or progressively fractionated by filtration, in each case the seric reactions are retained as long as the virus is present and active, they diminish in strength in a way closely parallel to the progressive loss in infectivity, and they disappear at just the point at which the virus becomes no longer demonstrable.

3. When tobacco-mosaic virus and potato latent-mosaic virus-immune sera are tested with their respective viruses propagated in hosts distantly removed systematically from the hosts used in serum preparation, the seric reactions are demonstrable, correlated with the amount of infective material and independent of the host plants used.

4. From these findings, as well as from the facts previously reported concerning virus neutralization, the possibility of arriving at a logical classification of viruses by means of serological reactions, and the correlation between strength of serological reactions and the amount of infective material present in test samples, it is concluded that the evidence now available is sufficiently strong to warrant the assumption that the antigens responsible for the plant-virus serological reactions thus far studied are the viruses themselves and not normal or derived constituents of diseased plants.

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THE EFFECT OF LIGHT ON THE INITIATION OF RUST INFECTION[1]

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Since Hart (2) reported that germ tubes of *Puccinia graminis tritici* Eriks. and Henn. did not enter wheat stomata except while the stomata were open during hours of sunlight and, since Caldwell and Stone (1) reported that germ tubes of *P. triticina* Eriks. seemed able to force entrance through the closed stomata of wheat seedlings, extensive experiments were planned to determine the effect of light during the early stage of the incubation period for several different rusts.

From Loftfield's work (3) and from our own observations it appears that almost all the stomata of cereals are closed in darkness, even in extremely high atmospheric humidity. If the germ tubes of cereal rusts enter their hosts during the hours of darkness it may be assumed that they make their way between the guard cells of closed stomata or that occasional stomata remain open, because they cease to function entirely or are clogged by dust particles, or are situated near the ends of veins and close to the water supply of the leaf. It also is possible that stomata open temporarily in darkness when there is an excess of CO_2 (see Scarth, 5). If germ tubes are able to penetrate closed stomata we might expect almost as great severity of infection when plants are in darkness as occurs under normal conditions of alternating light and darkness with long periods of high atmospheric humidity. If entry is through the occasional stomata that remain open we might expect the severity of infection occurring in darkness to be very much less than normal.

In the following experiments 2 physiologic forms of *P. graminis tritici* were used: form 21 on seedlings of the wheat varieties, Marquis C. I. 3641, Hope C. I. 8178, Acme C. I. 5284, Velvet Don C. I. 1445, Mindum C. I. 5296, Webster C. I. 3780, and Marquillo C. I. 6887, seedlings of the first 5 varieties being completely susceptible to this form while the other 2 are resistant; and form 49 on seedlings of Marquis, Velvet Don, Mindum,

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Hope, and Marquillo, the variety Marquis being susceptible and the others resistant to this form.

Puccinia triticina 53 was used, with seedlings of the completely susceptible Little Club C. I. 4066 wheat as hosts.

Seedlings of Gopher oats C. I. 2027 and Victory oats C. I. 1145 were inoculated with forms 1 and 17 of *Puccinia coronata* Cda. Both varieties are susceptible to both rust forms.

Puccinia sorghi Schw. was included in the experiments and was tested on young plants of the Golden Bantam variety of corn, *Zea mays* L.

In addition, 3 other rusts were used, although we were uncertain as to the distribution of stomata on their host plants and the behavior of the stomata in darkness. Young plants of *Phaseolus vulgaris* L. (Kentucky Wonder beans of commercial source) were inoculated with *Uromyces appendiculatus* Fries; cotyledons and seedling leaves of *Helianthus annuus* L. were inoculated with *Puccinia helianthi* Schw.; and plants from cuttings of *Antirrhinum majus* L. were inoculated with *P. antirrhini* D. and H.

The experiments were made in the winters of 1931–32 and 1932–33. The "light series" was run in the greenhouse at a temperature range of 18° to 25° C., averaging about 21° C. There were about 9 hours of daylight during the winter months, with slightly more in autumn and early spring. Some of the "dark series" were run in a photographic dark room where the temperature usually was 20–22° C. An electric fan circulated the air in the dark room and conditions remained fairly uniform and comparable with those of the light series throughout the experiments. Others of the dark-series experiments were made in a heavy zinc box, $4 \times 4 \times 3$ feet, tightly fastened and padded to keep out light, but with no facilities for circulation of air.

Most of the inoculations were made in the evening after dark, when we might be reasonably certain that the stomata of the cereals were closed. In some cases plants had been in darkness from 1 to 3 hours before they were inoculated; more often 5 to 6 hours elapsed before inoculation; and in some experiments the plants were kept in darkness 8 to 12 hours or longer before inoculation. In one case wheat seedlings were kept in the dark 48 hours previous to, and 56 hours after, inoculation, with no apparent injurious effect on the metabolism of the seedlings. With oats, however, a long period in darkness was detrimental to the development of the seedlings, which were etiolated so severely that some failed to recover when returned to light. By planning rather short periods of darkness previous to inoculation and not too long a period in the moist chamber, it was hoped to avoid the possibility of temporary opening of stomata in darkness that Scarth (5) suggested might take place in *Zebrina pendula* if CO_2 accumulated.

Abundant inoculum was obtained from stock cultures in the greenhouse and was applied to the moistened leaf surfaces by flattened needles or was rubbed on with the fingers. The inoculated plants were kept in moist chambers for varying lengths of time, usually 12 to 24 hours and occasionally as long as 72 hours. For the dark series the plants were in darkness throughout the moist-chamber period, and usually the plant surfaces were allowed to dry before the plants were returned to the light. In the light series conditions were more or less comparable to field conditions, the inoculated plants being placed in moist chambers during the night and then exposed to alternating periods of darkness and light. At the end of the moist-chamber period, plants were returned to the greenhouse bench until infection notes could be taken. In some cases 12 to 14 days elapsed before final notes were taken, but usually 10 days sufficed for the eruption and normal development of rust pustules, yet insufficient for secondary infection. The type of rust infection was recorded, for some hosts were completely susceptible and some were resistant. The prevalence of infection (the percentage of plants infected) was determined in all experiments, allowance being made for seedlings that emerged or leaf blades that expanded after inoculation. Severity of infection (the abundance and distribution of rust uredia over a leaf surface) also was determined according to scales set up for the different rusts, patterned after the scale used by the Division of Cereal Crops and Diseases for estimating rust percentages. Five classes were established, the 3 lowest constituting a light-infection group, the 2 highest forming a heavy-infection group. The average percentages of infection for the 3 lowest classes established were trace, 5 per cent, and 10 per cent. In the other 2 classes severity of infection averaged between 25 and 40 per cent for one class and between 55 and 80 per cent for the other.

In a few experiments attempts were made to study the stomatal movements of the inoculated plants, but removal of plants from moist chambers for stomatal observations usually reduced the relative humidity of the atmosphere and interfered with the proper incubation of the inculated plants.

THE PREVALENCE OF RUST INFECTION IN LIGHT AND IN DARKNESS

In all the experiments accurate counts were made of the number of plants infected and the number inoculated (Table 1). The prevalence of infection of all rusts except *Puccinia graminis* varied from 82.8 per cent to 100 per cent when the plants were incubated in the light. Under the same conditions the prevalence of *P. graminis* was somewhat lower, ranging from 70.0 to 90.1 per cent, but being as low as 54.8 per cent when the resistant Hope wheat was inoculated with form 49. In general, however,

TABLE 1.—*The effect of light during inoculation and the early stages of infection on the prevalence and severity of infaction for seven rust species*

Rust	Host	Light conditions in moist chamber	Prevalence of infection			Severity of infection	
			Number of plants inoculated	Number of plants infected	Percentage	Percentage plants with light infection	Percentage plants with heavy infection
Puccinia triticina	Little Club wheat	Light	157	131	83.4	19.1	64.3
		Dark	389	342	87.9	3.8	84.1
P. antirrhini	*Antirrhinum majus*	Light	782	648	82.8	44.6	38.2
		Dark	392	358	91.3	39.9	51.5
P. coronata	Gopher oats	Light	75	73	97.3	40.0	57.3
		Dark	165	163	98.7	36.3	62.4
"	Victory oats	Light	377	335	89.0	12.8	76.2
		Dark (Short)	135	125	92.6	33.3	59.3
		Dark (Long)	236	134	56.7	56.7	0.0
P. helianthi	*Helianthus annuus*	Light	106	106	100.0	16.0	84.0
		Dark	58	54	93.1	24.1	69.0
P. sorghi	*Zea mays* (Golden Bantam)	Light	436	395	90.6	24.3	66.3
		Dark	231	164	70.9	31.6	39.3
Uromyces appendiculatus	*Phaseolus vulgaris* (Kentucky Wonder)	Light	68	60	88.2	27.9	60.3
		Dark	60	46	76.6	43.3	33.3
P. graminis tritici 21	Marquis wheat	Light	440	341	77.5	6.1	71.4
		Dark	535	281	52.5	40.4	12.1
	Hope	Light	435	348	80.0	5.1	74.9
		Dark	436	256	58.7	28.0	30.7
	Acme	Light	91	71	78.0	8.8	69.2
		Dark	67	42	62.7	38.8	23.9
	Velvet Don	Light	235	169	71.9	4.7	67.2
		Dark	215	116	53.9	20.9	30.0

TABLE 1.—(*Continued*)

Rust	Host	Light conditions in moist chamber	Prevalence of infection			Severity of infection	
			Number of plants inoculated	Number of plants infected	Percentage	Percentage plants with light infection	Percentage plants with heavy infection
P. graminis tritici 21	Mindum	Light	81	73	90.1	0.0	90.1
		Dark	80	60	75.0	16.3	58.7
	Webster	Light	39	34	87.2	53.9	33.3
		Dark	50	17	34.0	34.0	0.0
	Marquillo	Light	313	220	70.2	9.2	61.0
		Dark	324	191	58.9	19.7	39.2
P. graminis tritici 49	Marquis	Light	216	172	79.6	17.6	62.0
		Dark	248	95	38.3	26.2	12.1
	Velvet Don	Light	191	150	78.5	15.7	62.8
		Dark	152	81	53.3	26.3	27.0
	Mindum	Light	209	153	73.2	13.9	59.3
		Dark	232	139	59.9	17.2	46.7
	Hope	Light	135	74	54.8	32.6	22.2
		Dark	147	42	28.5	24.5	4.0
	Marquillo	Light	414	290	70.0	26.1	43.9
		Dark	347	209	60.2	23.9	36.3

with all the rusts concerned, a large proportion of inoculated plants became infected.

When plants were inoculated in darkness and darkness prevailed throughout the moist-chamber period, the prevalence of infection usually differed from that obtained on plants in the light. With *P. graminis tritici* it was considerably less than in light—from 9.8 to 53.1 per cent less, depending upon the rust form and the host. In the variety Marquis, completely susceptible to both rust forms, the reduction in prevalence of infection in darkness was 25.0 per cent with *P. graminis tritici* 21 and 41.3 per cent with form 49. In the resistant Marquillo, on the other hand, the reductions were 11.3 and 9.8 per cent, respectively.

With *Puccinia sorghi* the prevalence of infection in darkness was nearly 20 per cent less than in light, and with *Uromyces appendiculatus* there was a reduction of 11.6 per cent. However, darkness seems not to reduce the prevalence of infection of some other rusts. With *Puccinia triticina* the prevalence of infection was actually slightly higher in darkness than it was in light (4.5 per cent higher), while for *P. antirrhini* the prevalenec of infection in darkness was 8.5 per cent higher than that in light.

In the case of *Puccinia coronata* darkness did not hinder infection, the prevalence of infection on Gopher oats in darkness being approximately the same as in light, and the prevalence on Victory oats being slightly higher in darkness than in light. However, darkness in excess had a decided effect on Victory oats seedlings and the prevalence of crown-rust infection. After 18 to 48 hours in darkness before inoculation and 48 to 60 hours in darkness after inoculation oats seedlings were severely etiolated and on many the tips of the leaf blades were dying. The tissues exposed to rust infection were impoverished, some temporarily, others permanently. The prevalence of crown-rust infection on such plants was reduced to 56.7 per cent, and most of the infections were in the basal region of the leaf blade, the part that recovered first from the great etiolation. The results of these experiments probably are to be attributed to the effect of darkness on host metabolism, not to its effect on stomatal behavior and entry of the parasite.

THE SEVERITY OF RUST INFECTION IN LIGHT AND IN DARKNESS

Perhaps more important than the prevalence of rust infection is the severity of infection: the abundance and distribution of rust pustules over the leaf surface. If the rust inoculum is viable and virulent and has access to its host, it may be expected to cause heavy infection such as is shown in figure 1, A. On Hope and Marquis, the two hosts completely susceptible to form 21 and incubated in the light, the infection centers are numerous and distributed over almost the entire leaf surface and occupy a relatively large proportion of the leaf area. Even on the resistant Mar-

quillo host, in spite of the fact that less of the leaf area seems to be affected, the severity of infection is high, since there are 51 infection centers as compared with 58 and 70 for the 2 completely susceptible hosts. If light has no effect on the entry of rust or the very early stages of infection, we might expect similar severity of infection in light and in darkness. But if the host stomata are closed in darkness and the rust cannot enter its host through closed stomata, we might expect a relatively light infection (Fig. 1, B) or merely a trace of infection. Lack of light reduces the severity of infection by some rusts but has no effect in the case of others.

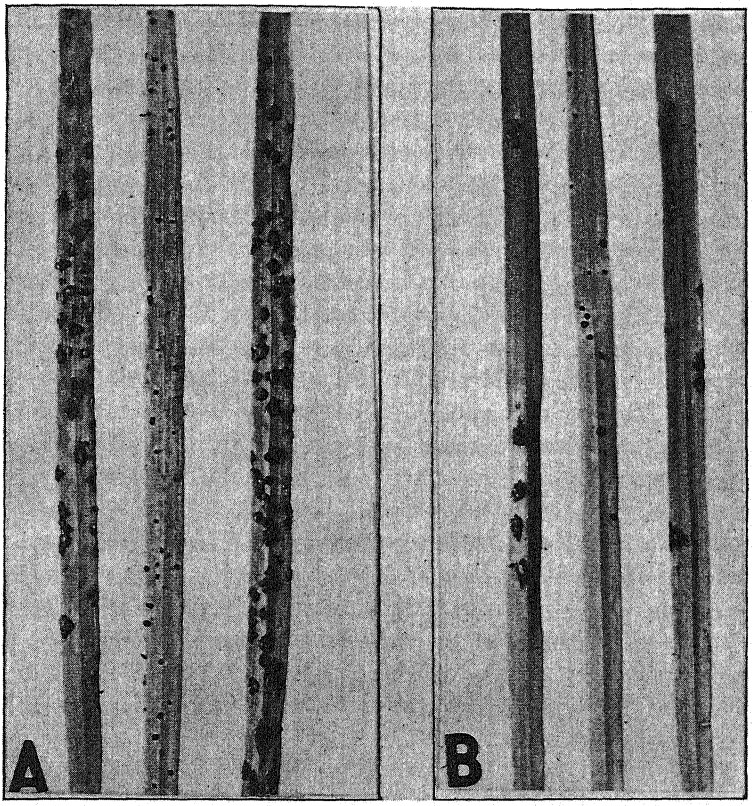


FIG. 1. A. *Puccinia graminis tritici* 21 on seedlings of Hope, Marquillo, and Marquis, inoculated and incubated in the light. There are 58, 51, and 70 infection centers, respectively, even though the resistant host Marquillo has a 2^{+c} type of infection while the susceptible Hope and Marquis have pustules of the 4^{+} type. B. Form 21 on the same varieties, inoculated and incubated in the dark, with 5, 14, and 8 infection centers, respectively.

Most of the seedlings of Little Club wheat rusted by *Puccinia triticina* were severely infected, falling in the 2 highest classes established for infec-

tion severity. Less than 4 per cent of the seedlings that had been inoculated and incubated in the dark escaped with light infection. Thus, as Caldwell and Stone (1) pointed out, darkness during inoculation and throughout the moist-chamber period does not inhibit leaf-rust infection of wheat. The same is true of *P. coronata* on Gopher oats, most of the rusted plants being severely infected whether they were inoculated and incubated in light or in darkness. Some oats plants escaped with light infection, but more than half were severely rusted. Nearly all the wheat plants were severely rusted by *P. triticina*. *Puccinia antirrhini*, the snapdragon rust, also, was not deterred by darkness from infecting its host leaves heavily. As a matter of fact darkness favored severe infection somewhat more than did light. The majority of the plants in darkness were severely rusted, while in light the majority of the plants were lightly rusted.

In the experiments with stem rust of wheat our results are at variance with those of Peterson (4), for in our work darkness reduced the severity of infection in most varieties. As a general rule the majority of plants inoculated and incubated in light were severely rusted, while relatively few were lightly rusted. For example, 71 per cent of the Marquis seedlings inoculated and exposed to light were heavily rusted by *P. graminis tritici* 21 and only 6 per cent were lightly rusted (Table 1). Many of the seedlings of other varieties were rusted as heavily as Marquis was, but there were 2 exceptions: Form 21 produced light infection on the majority of the resistant Webster plants inoculated and incubated in the light; and Hope, resistant to form 49, was more often lightly infected than severely infected when incubated in the light. On susceptible varieties, nevertheless, the majority of plants were severely rusted.

The effect of darkness at inoculation and during the moist-chamber period was to shift the severity of infection towards the lower classes so that the proportion of plants with light infection was increased, sometimes to such an extent that most of the rusted plants were only lightly infected, whereas, in light, the majority had been severely infected. Thus, 40.4 per cent of Marquis seedlings inoculated with form 21 and incubated in the dark were only lightly rusted, while 12.1 per cent were severely rusted (Table 1), thus increasing the percentage of plants with light infection from 6.1 (in light) to 40.4 (in darkness) and decreasing the percentage with heavy infection from 71.4 (in light) to 12.1 (in darkness).

In other cases the shift was not so decided. In the light, Hope infected with form 21 was very much like Marquis—74.9 per cent of the seedlings were severely rusted and only 5.1 per cent lightly rusted. In darkness, however, about half, or slightly more, of the rusted seedlings were severely infected and half lightly infected; that is, 30.7 per cent of the seedlings in darkness were severely rusted as compared with 74.9 per cent severely

rusted in light, while 28.0 per cent in darkness were only lightly rusted as compared with 5.1 per cent in light. In Mindum, on the other hand, when *Puccinia graminis tritici* 49 is used, the change in proportions is very slight. Darkness reduces the percentage of severe infection from 59.3 to 46.7, while the percentage of plants with light infection is increased slightly, from 13.9 to 17.2. The one exception to this general reaction to darkness is found in Marquillo when rusted by *P. graminis tritici* 49. The proportions of lightly rusted and severely rusted plants are approximately the same in light and in darkness.

Darkness had a similar effect on the development of bean rust. Sixty per cent of the bean leaves incubated in the light were severely rusted by *Uromyces appendiculatus,* but in darkness only 33 per cent were severely rusted. The percentage of leaves with light infection was nearly 28 in light but darkness increased this to 43.3 per cent, so that in darkness the majority fell into the light-infection group.

Infections by *Puccinia sorghi* and *P. helianthi* were affected by light, but to a less extent than infections by *P. graminis* and *Uromyces appendiculatus.* Darkness reduced the percentage of plants with severe infection but in no case shifted the severity of infection so much that the majority of the plants were only lightly rusted. Sixty-nine per cent of the sunflowers and 39.3 per cent of the corn plants inoculated and incubated in the dark were severely rusted—and these figures represent majorities of the rusted plants.

Light or its absence did not affect the severity of crown rust on Gopher oats, but on Victory oats darkness slightly reduced the proportion of severely rusted plants and increased the proportion of lightly rusted plants. Darkness in excess resulted in very light rust infection, but, as discussed previously, this was to be attributed to the etiolation and poor vigor of the host.

DISCUSSION

The question of initiation of rust infection during darkness may have a bearing on the opportunities for primary infection and spread of rust in the field, and it may be concerned in the apparent ease with which an outbreak of one rust develops to epidemic proportions while epidemics of other rusts seldom occur or are secured with difficulty by artificial means.

Moisture is the first requisite for spore germination and the growth and survival of germ tubes. If it is the only requisite up to the time the rust sends its haustoria into the host cells, infection might occur whenever moisture was available for short periods—during rains or a series of showers, or when the plants were covered with dew for a few hours. But rust germ tubes enter their graminaceous hosts through the stomata, which

usually are closed in darkness. Some rusts are not impeded by closed stomata, so that moisture still may be considered the prime requisite for the functioning of inoculum and initiation of infection. Other rusts are unable to penetrate closed stomata; consequently there are 2 requisites to be considered: moisture and stomatal openings. Opportunities for infection are somewhat more limited, for it occurs only during the time when there is sufficient moisture and stomata are open. Darkness hinders infection by rusts of this class because most of the stomata close in darkness.

Leaf rust of wheat, crown rust of oats, and snapdragon rust seem to enter their hosts equally well in light or in darkness. On the other hand, darkness hinders greatly the infection of wheat by *Puccinia graminis* and of beans by *Uromyces appendiculatus,* and darkness deters slightly the infection of corn and sunflowers by their respective rusts.

Under some field conditions the restrictive action of darkness may not be apparent and may be of no importance in the initiation of infection and spread of rust. If inoculum is abundant at all times and environment favors continual inoculation and infection, the final amount of rust may be as great and as overwhelming as though darkness had no effect on the entrance of the fungus. But if the inoculum enters an area in waves and environment favors infection only at short intervals during the growing season, the limiting effect of darkness may be sufficient to reduce the amount of infection considerably.

SUMMARY

Experiments were made to determine the effect of light and of darkness on the entry of uredial germ tubes and development of *Puccinia triticina, P. graminis tritici, P. coronata, P. sorghi, P. helianthi, P. antirrhini,* and *Uromyces appendiculatus.*

Darkness at the time of inoculation and throughout the early stages of infection reduced the prevalence and severity of infection by *P. graminis tritici* and *Uromyces appendiculatus.*

Darkness had no effect on the prevalence and severity of infection by *P. triticina* or *P. antirrhini.*

The prevalence of infection by *P. sorghi* and *P. helianthi* was reduced by darkness; and the severity of infection was reduced slightly, but not sufficiently to place the majority of infected plants in the "light infection" class.

Darkness did not affect the prevalence of infection by *P. coronata,* nor the severity of its infection on Gopher oats; but on Victory oats the severity of infection by *P. coronata* was reduced slightly.

The effect of darkness on infection by *P. graminis tritici* varied somewhat according to the wheat variety and rust form used. Infection of the

susceptible variety Marquis was decreased decidedly by darkness, but there was less effect on the infection of the resistant variety Marquillo.

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PHYTOPATHOLOGICAL NOTES

An Internal Necrosis of Bean Seeds.[1]—During the early spring of 1934 some bean seeds were sent to the Department of Biology by W. D. Click, the county agent of Wayne County. These seeds showed brown necrotic spots in the center of the flat inner surface of both cotyledons. There was no external symptom in evidence. These seeds represented a sample taken from a lot that had been purchased for planting by the Relief Administration of Wayne County. They were purchased from H. D. Woodruff and Sons of Orange, Conn., according to advice from Mr. Click. It is not known where they were grown.

Because the disease appeared to be unrecorded in the literature on beans, it seemed advisable to follow it up; consequently a larger sample was secured for planting in the pathologium. No count of germination was taken, but a fair stand was secured and a small crop of pods harvested. At this time it was noted that two varieties[2] were represented in the planting. The broad pod variety was determined as Wooster Mammoth and the narrow pod variety as Jarvis. The markings on the surface of the seeds are similar, but are slightly lighter on Jarvis.

An examination was made of 100 of the harvested pods and the condition of every seed in each pod recorded. The Wooster Mammoth variety showed a very high percentage of this defect. Forty-seven out of 49 pods contained one or more seeds showing the necrotic spot, and in 33 of these pods all seeds were defective. The Jarvis variety appears to be less susceptible. Only 10 pods out of 51 examined showed any defect and in only 2 cases were all seeds in the pod spotted.

Several attempts were made to isolate an organism from the spots. The necrotic area was removed aseptically and placed in sterile broth medium, but no evidence of any associated organism was found. Apparently the disturbance is of a non-parasitic nature and more work is essential before any definite statement can be made.

The only disease known to the writers that bore resemblance to this internal necrosis is the "Marsh Spot" of peas as described by De Bruijn[3] and attributed by her to climatic and soil conditions. Recently, Zaumeyer

[1] Published with the approval of the director, West Virginia Agricultural Experiment Station, as Scientific Paper No. 150.

[2] The pods were submitted to the Bureau of Plant Industry, U. S. Department of Agriculture and identification made by Dr. Victor C. Boswell.

[3] De Bruijn, H. L. G. Kwade harten van de erwten (Marsh Spot of Peas) Tijds. Plantenziek. 39: 281–318, 1933.

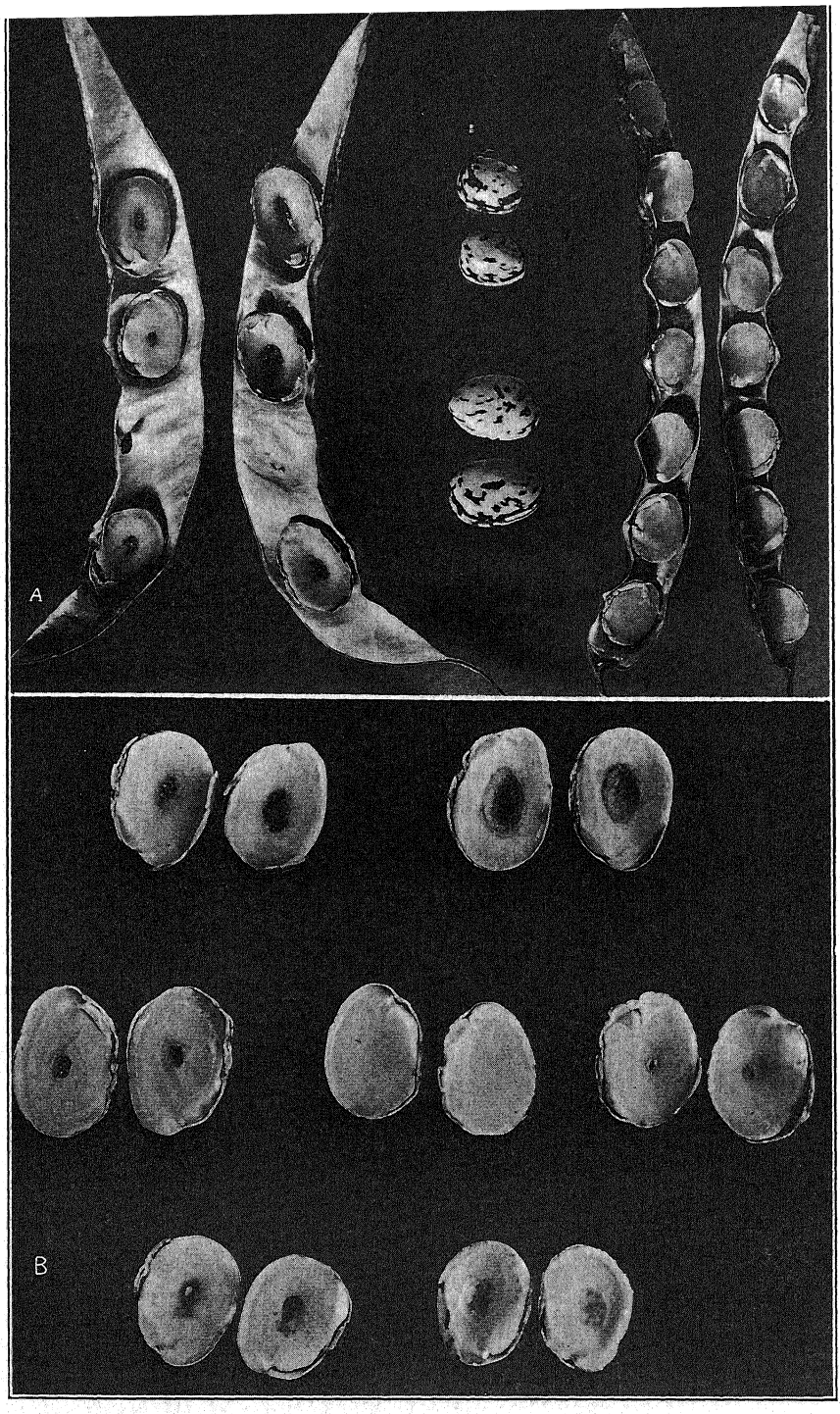


FIG. 1. A shows the difference in size of pods and seeds of the two varieties. The larger is the Wooster Mammoth, the smaller the Jarvis. Spotting of the larger variety is very clear. The seeds of the smaller variety show only a very slight defect in one cotyledon, the second from the bottom. B shows several affected seeds. The spots vary in color from a dark brown to a pale yellow and in size from very small markings to discolorations that may cover half the inner surface of the cotyledons. The seed in the center of the figure is healthy.

and Wade[4] have reported a similar internal spotting in western peas and under greenhouse conditions at Rosslyn, Virginia.

In both peas and beans the defect has every appearance of being non-parasitic unless there be a virus concerned. However, it appears that in the case of the bean necrosis there may be heritable tendencies that influence its incidence. Further studies are necessary to clear up these problems.—C. R. ORTON and W. D. HENRY, University of West Virginia, Morgantown, W. Va.

Filtrability of Certain Plant Pathogenic Bacteria.—Phytomonas tumefaciens (Sm. and Town.) Stevens and *Bacterium gypsophilae* Brown are the only phytopathogenic bacteria the filtrability of which seems to have been reported. Rosen[1] observed in young bouillon cultures of *P. tumefaciens* buds that passed through Berkefeld V filters. Lieske[2] reported filtrability of old bouillon cultures of *P. tumefaciens* and not of cultures grown in milk. Schätzel[3] was able to get a filtrable form of *P. tumefaciens* in old as well as in young cultures whether grown in bouillon or milk. Brown[4] established filtrability of *B. gypsophilae* and *P. tumefaciens* when grown in beef bouillon and filtered through Chamberland L_3 filters.

The writer, working on methods of procuring toxins from *Erwinia amylovora* (Burrill) Winsl. *et al.*, observed that some filtrates produced growth in bouillon which appeared to be a pure culture of *E. amylovora*. Investigation, therefore, was undertaken to study filtrability of *E. amylovora*. Tubes of common nutrient broth inoculated with *E. amylovora* were incubated at 28° C. and then filtered through new Berkefeld V- and N and Chamberland L_3 filters at different intervals of time. Filtrates of these cultures tested up to 2 months remained sterile. On the other hand, skimmed milk used as a medium was found to induce a filtrable stage of *E. amylovora*. A large number of tubes of sterile milk inoculated with *E. amylovora* and incubated at 28° C. was filtered and cultured after 7, 12, 24, and 36 days, respectively. The above named filters were used. *Bacillus prodigiosus* (Ehrenb.) Flügge and *Bacterium coli* (Escher.) Lehm. and Neum. always were mixed with the cultures to be filtered to check the efficiency of the filters used. After filtration 1 cc. of the filtrate was transferred into nutrient broth of pH 6.9 and incubated at 28° C. At the same

[4] Zaumeyer, W. J., and B. L. Wade. Physiological spotting of pea seed. Phytopath. 24: 1383–1384, 1934.

[1] Rosen, H. R. Morphological notes together with some ultrafiltration experiments on the crown-gall pathogene, *Bacterium tumefaciens*. Mycologia: 18: 193–205. 1926.

[2] Lieske, Rudolph. Untersuchungen über die Krebskrankheit bei Pflanzen, Tieren und Menschen. Centralbl. f. Bakt. etc. Abt. I. Originale 108: 118–146. 1928.

[3] Schätzel, Karl. Beiträge zur Morphologie und Physiologie des bakteriellen Pflanzenkrebserregers. Phytopath. Zeitschrf. 5: 251–273. 1933.

[4] Brown, Nellie A. A gall similar to crown gall, produced on Gypsophilla by a new bacterium. Jour. Agr. Res. 48: 1099–1112. 1934.

time potato-dextrose-peptone agar plates were streaked with a loopful of filtrate and subsequently incubated at 28° C. Agar plates developed no visible colonies in any case, while tubes with nutrient broth became turbid after 48 hours of incubation. Table 1 presents data on filtration of the milk cultures. It is apparent that the particles that constitute a filtrable form of the fire-blight organism can pass through Berkefeld V and N and Chamberland L_3 filters.

TABLE 1.—*Filtrability of Erwinia amylovora grown in skimmed milk*

Kind of filter	Incubation period (days):							
	7		12		24		36	
	Number of tubes:							
	+	−	+	−	+	−	+	−
Berkefeld V	10	0	10	0	10	0	12	0
Berkefeld N	10	0	10	0	10	0	10	2
Chamberland L_3	10	0	15	0	12	3	25	1

Smears of *Erwinia amylovora* made from the milk culture, when stained with thionin and methylene blue, revealed organisms with very dark-stained, round polar bodies. After 24 days, long chains were numerous in the cultures. All cultures obtained from the filtrates were found to be pathogenic on green pear fruits.

The same type of experiment was performed with the soft-rot organism, *Erwinia carotovora* (L. R. Jones) Holland. In the case of this organism the filtrability, again absent in bouillon, was demonstrated when the organism was grown in milk. As soon as the organism produced a considerable quantity of acid as a result of splitting milk sugar, no filtrability could be detected. Although the exact pH at which filtrability disappears was not determined, it was noted that cultures that had reached pH 6.6 no longer yielded filtrable forms.

Thus it appears that the filtrability of *Erwinia amylovora* and *E. carotovora* may be obtained under laboratory conditions by use of a special medium. Hydrogen-ion concentration of the medium may be a limiting factor in this phenomenon.

Attempts to get a filtrable stage of the 2 mentioned organisms from parts of the plants affected by them were unsuccessful.—P. A. ARK, Division of Plant Pathology, University of California, Berkeley, California.

Soggy Breakdown of Winter Banana Apples.[1]—Attention has been called to the similarity of the diseases known in the literature as soft scald and soggy breakdown of apples, as these occur on the varieties Wealthy and Golden Delicious.[2, 3, 4] In these two varieties the superficial appressed blemished areas typical of the soft-scald type of the disease may be found not only in the same storage lots of fruit, but on specimens that also have the typical internal deep-seated, soggy breakdown areas within the cortex region. That these two types of breakdown occur in the same storage lots under similar treatment and simultaneously on the same as well as in different specimens, suggests that they are identical.

Additional evidence indicating that this is the case has been obtained by recent storage experiments with the variety Winter Banana. In these experiments the apples were harvested on the usual commercial date and handled and stored in much the same manner as in investigations previously reported.[2, 5] Some lots of fruit were stored within 24 hours of picking, while others were delayed at 4-day intervals at a temperature of 50° F. before storing at 31° and 36° F.

All lots of fruit developed the low-temperature breakdown at 31° F., but those stored immediately and within the 5- and 9-day interval after picking were much more affected than those given longer delayed treatment. As with other varieties in other studies, no soggy breakdown occurred on this variety at 36° F. These results are similar to those obtained previously with Golden Delicious and Grimes Golden.[2, 5]

The appearance of the two types of breakdown occurring in this variety is shown in figures 1 and 2. In figure 1 is shown the broken down areas of the cortical portion of the apple, which are typical for the soggy breakdown type earlier described on Grimes Golden (*loc. cit.*). Figure 2 shows the superficial type of the disease, with the characteristic appressed and sometimes depressed blemishes, which resembles the soft-scald type common on Jonathan, Golden Delicious, Wealthy, and Northwestern Greening. Transverse and longitudinal sections of affected apples, however, (figure 2) frequently exhibit the same deep-seated broken-down areas as those illustrated in figure 1. Moreover, the superficial areas frequently coalesce with the deeper-seated soggy breakdown areas. The question then arises as to

[1] Journal Paper No. J-259 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 279.

[2] Plagge, H. H., and Maney, T. J. Soggy breakdown of apples and its control by storage temperature. Iowa Agr. Exp. Sta., Res. Bul. 115. 1928.

[3] Plagge, H. H. A study of soggy breakdown and some related functional diseases of the apple. Proc. Amer. Soc. Hort. Sci., 26: 315–318. 1929.

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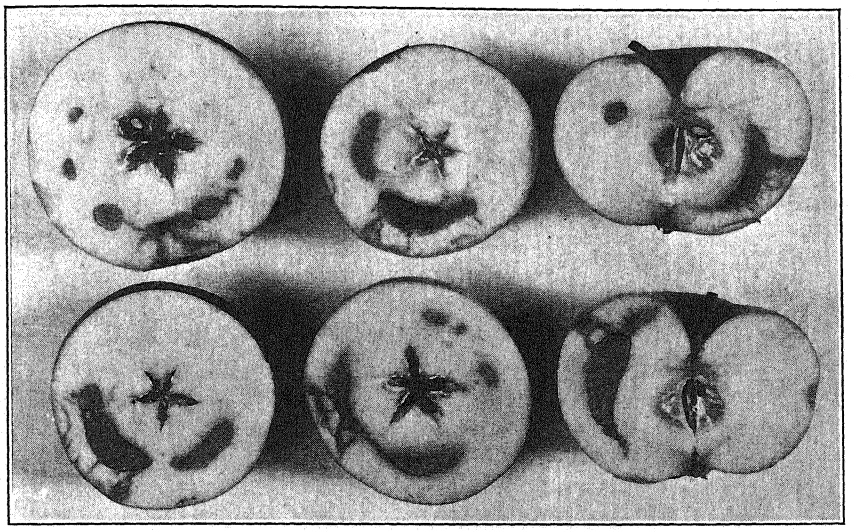


FIG. 1. Soggy breakdown in Winter Banana Apple.

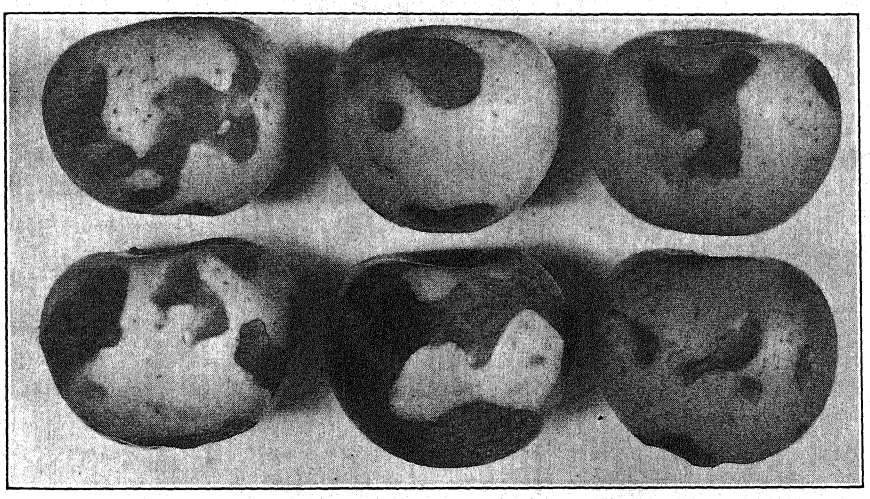


FIG. 2. Soft scald type of soggy breakdown of Winter Banana Apple.

the limitations of each type of disorder. The appearances of the diseased tissue of both types of the disorder, in all the varieties thus far studied, are similar in color, taste, and consistency. The results of this study with Winter Banana and other varieties (*loc. cit.*) have led the authors to conclude that the two types of low-temperature diseases herein illustrated and described are identical.—H. H. PLAGGE and T. J. MANEY, Department of Horticulture, Iowa State College, Ames, Iowa.

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FUSARIUM STRAINS IN RELATION TO WILT OF CHINA ASTER[1]

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INTRODUCTION

The present publication, an abstract of which has already appeared (25), describes studies at the University of Wisconsin on the pathogenicity to China aster, *Callistephus chinensis* Nees, of various Fusarium strains from this country and abroad. These include both Fusaria that produce wilts of other hosts and Fusaria isolated from wilting asters. In addition, the behavior of the pathogenic forms has been tested toward host strains of known character, *i.e.*, not only toward those susceptible but also toward those resistant to the Beach and the Jackson isolations of *Fusarium conglutinans* Wr. v. *callistephi* Beach.

This paper is in continuation of one by Jones and Riker (13) dealing with the nature and control of the wilt and yellows of the China aster. That publication confirms the earlier work of Beach (4) and others that the wilt, caused by the vascular Fusarium, *F. conglutinans* v. *callistephi,* is wide spread and destructive in the United States, and indicates that, like other vascular fusarium diseases, this aster wilt yields to control through the selection of wilt-resistant host strains. As therein detailed, selections for wilt resistance against the Beach and the Jackson isolations of *F. conglutinans* v. *callistephi* were stabilized to the point of commercial acceptance in several basic colors and a fairly wide range of flower type and growth habit. Particular attention was paid to the American Branching type, most in use by the florists and home gardeners of the Wisconsin-Chicago district, in the important florists' colors, *viz.*, white, pink, rose, lavender, purple, and red, and to the Heart of France. Enough additional work was done with several other types of China aster (Royal, Comet, and American Beauty) to justify the conclusion that, in general, by repeated selection a commercially satisfactory degree of resistance may be found to this fusarium wilt. It also was pointed out in the same publication that these selected strains proved resistant not only in Wisconsin, but in such

[1] Published with the approval of the Director of the Wisconsin Agricultural Experiment Station.

widely scattered localities in this country as Washington, D. C., Yonkers, New York, and El Monte, California. Favorable reports on the behavior of the strains in Ithaca, New York (22, 23, and 24), and West Chicago, Illinois (2), have appeared in the literature.

PATHOGENICITY OF VARIOUS FUSARIUM STRAINS TO CHINA ASTER

Pathogenicity of Various Vascular Fusaria that Induce Wilts of Other Hosts

The serious wilt-inducing Fusaria are, in general, placed by Wollenweber[2] in one section, *Elegans*. Because of the mutual resemblances of these pathogens, as well as of the associated diseases, it seemed worth while to test the pathogenicity to China aster of as many of these from other hosts as were available. Three series of trials were made, all in greenhouse culture. For these, seedlings of an aster strain susceptible to the Beach and the Jackson isolations of *F. conglutinans* v. *callistephi* were grown in flats of wilt-free soil. When the second leaf appeared, they were transplanted to 5-inch pots, 6 to each pot. At the time of transplanting the soil was inoculated by placing a small section of an agar plate culture of the organism to be tested in the hole into which the plant was dropped. In every series 2 pots were used for each organism. Thus, in all, 36 plants were tested with each Fusarium. The Fusaria of the section *Elegans* used,[3] with their classification and host source, were:

Sub-section *Orthocera*
- *F. conglutinans* Wr. from cabbage
- Do. ..... v. *betae* Stew. from sugar beet
- *F. orthoceras* App. et Wr. v. *pisi* Wade from pea

Sub-section *Constrictum*
- *F. bulbigenum* Cke. et Mass. v. *niveum* (Erw. Sm.) Wr. from watermelon
- Do. ..... v. *tracheiphilum* (Erw. Sm.) Wr. from cowpea

Sub-section *Oxysporum*
- *F. oxysporum* Schl. from cabbage
- Do. ..... beet
- Do. ..... potato
- *F. vasinfectum* Atk. from cotton

In addition, an unidentified Fusarium from celery was used.

In these trials, none of the organisms tested induced wilt of China aster. With each of the organisms listed above, the plants remained 100 per cent

[2] The nomenclature and classification of the Fusaria used throughout this paper are those of Wollenweber (40).

[3] The cultures employed were furnished by various members of the Department of Plant Pathology at the University of Wisconsin with the following exceptions. The 3 strains of *Fusarium oxysporum* were supplied by H. W. Wollenweber. *F. vasinfectum* was sent by V. H. Young. The assistance of these colleagues is gratefully acknowledged.

healthy at the end of 6 weeks, while in the controls, inoculated with the Beach isolation of *F. conglutinans* v. *callistephi,* only 8 per cent of the plants were healthy at the end of the same period.

These negative results on China aster confirm previous reports in the cases of *F. conglutinans* (11, 12, and 31) and *F. conglutinans* v. *betae* (31).

Pathogenicity of Various Fusaria from China Aster

A survey of the literature, supplemented by observations of L. R. Jones and by correspondence, indicates the almost universal occurrence of fusarium aster wilt wherever the culture of the host plant has been long established. Its widespread distribution in the United States has been indicated by the writers (13). In Canada the disease has been particularly studied in Ontario (11) and New Brunswick (15). In Europe it has been reported from Austria (7); Czecho-Slovakia (30) and (3); Denmark (9); England (18) and (19); Germany (many references including those of Gante (8) and Wollenweber (41)); Greece (1); Holland (26) and (32); Italy (21), (33), and (34); and Switzerland (20) and (16). In the summer of 1930 L. R. Jones observed what appeared to be fusarium wilt of aster under the guidance of H. W. Wollenweber in Germany, and Elias Melin in Sweden. The disease has been reported also from South Africa (35), (36), and (37); New Zealand (14) and (5); and Tasmania (17). In Australia a wilt of asters has been noted (6); and D. B. Adam, in correspondence, has referred to a wilt in China asters caused, he believed, by *Fusarium conglutinans* v. *callistephi.* In 1931 L. R. Jones was shown evidence of fusarium wilt in Japan by T. Fukushi and T. Hemmi.

The identity of the Fusarium involved has not always been determined. Several species besides *Fusarium conglutinans* v. *callistephi* have been listed as associated with diseased asters. However, the pathogenicity of many of these species has not been established. Wollenweber (39) listed, as found on China aster, *F. flavum* (Fr.) Wr., *F. dimerum* Penz., *F. orthoceras* Ap. et Wr., *F. conglutinans* Wr., *F. polymorphum* Matr., *F. culmorum* (W. Sm.) Sacc., *F. graminum* Cda., and *F. pyrochroum* (Desm.) Sacc. He pointed out that it had not yet been determined which of these were harmful. Gante (8) reported the isolation of *F. graminum, F. polymorphum, F. culmorum, F. falcatum* Ap. et Wr., and *F. dimerum* from diseased asters. He added that inoculation experiments were tried with *F. graminum, F. culmorum,* and *F. falcatum* and negative results obtained. Besides *F. conglutinans* v. *callistephi,* Jackson (11) found four other Fusaria, isolated from diseased asters in Canada, pathogenic to the host. Only one of these was identified, namely, *F. angustum* Sherb. *F. culmorum* isolated from asters was found nonpathogenic to asters. White (38), in considering the wilt of the carnation, reported that strains of *F. culmorum*

pathogenic to carnations caused aster wilt. Baudys (3) stated that the fusarium disease of asters in Czecho-Slovakia was ordinarily caused by *F. pyrochroum* and *F. conglutinans* v. *callistephi*. However, H. W. Wollenweber identified *F. solani* (Mart.) v. *Martii* (App. et Wr.) f. *1* Wr. and *F. scirpi* Lamb. et Fautr. v. *acuminatum* (Ell. et Ev.) Wr. on specimens sent to him. Wager (36) established *F. conglutinans* v. *majus* Wr. as the cause of aster wilt in South Africa. Wollenweber (41) mentioned *F. conglutinans* v. *majus* and *F. oxysporum* Schl. f. *6* Wr. as now and then isolated from wilted asters in Germany. He also listed *F. culmorum, F. sambucinum* Fckl., *F. lateritium* Nees, and *F. herbarum* (Cda.) Fr., with varieties, as observed on aster with harmful effect yet to be determined.

In the present studies the pathogenicity to China aster of various strains of Fusaria isolated from diseased asters was tested in the greenhouse and to a lesser extent in out-door cloth-covered houses. The strains of Fusarium used came from Europe and Japan and were compared in behavior with two American isolations of *F. conglutinans* v. *callistephi,* namely, the Beach isolation from Michigan and the Jackson isolation from Ontario.[4]

To test the pathogenicity of these Fusaria in the greenhouse, the method described in the preceding section was modified slightly. The seedlings were transplanted to flats instead of pots, each flat containing 48 plants. In the cases where negative results were obtained the trials were repeated twice, thus testing 144 plants with each organism. When positive results were obtained, further studies were conducted as described in the following section. Out-door trials in cloth-covered enclosures of certain of the Fusaria were made during the summer of 1931, using 50 plants with each organism.

The results of both greenhouse and out-door, cloth-covered-cage tests are summarized in table 1. In every instance the cloth-covered-cage results agreed with those obtained in the greenhouse. This table shows that, in the case of both the European and the Japanese Fusaria, some forms isolated from diseased asters proved pathogenic, while some did not. Of the pathogenic forms all but one, namely, *F. lateritium* Nees v. *fructigenum* (Fr.) Wr., belonged in the section *Elegans*. However, not all of the Fusaria of this section, isolated from diseased asters, proved pathogenic to aster.

Comparison with the results of the preceding section indicates that Fusaria, closely related morphologically and culturally, differ markedly in

[4] The writers wish to express their appreciation of the kind cooperation of several colleagues. The Beach and the Jackson isolations were sent by these men, respectively, and are the same as those used in the investigations reported in the earlier publication (13). The European forms were furnished and identified by H. W. Wollenweber. The Japanese forms were sent by T. Fukushi (J. A.) and T. Hemmi (J I, J II, J III, J VI), and the pathogenic strains identified by H. W. Wollenweber.

TABLE 1.—*Trials of pathogenicity to China aster of strains of Fusaria from wilting asters*

Fusaria		Pathogenicity[a]	
Species and culture number[b]	Source	Greenhouse	Outdoor cage
Elegans			
Orthocera			
F. conglutinans Wr. v. *callistephi* Beach (305)	Michigan	+	+
Do. (435)	Ontario	+	+
Do. (1472)	Germany	+	+
Do. v. *majus* Wr. (3206)	do	+	+
Do. (4422)	do	+	...
Do. (J. A)	Japan	+	+
Do. (J. VI)	do	+	+
Oxysporum			
F. oxysporum Schl. f. *6* Wr. (4179)	Germany	+	+
Do. (4200)	do	+	...
Do. (4497)	do	+	...
Do. (4506)	do	+	...
Do. (J. I)	Japan	+	...
Do. v. *aurantiacum* (Lk.) Wr. (4453)	Germany	0	...
F. redolens Wr. (4428)	do	0	...
Do. (4495)	do	0	...
F. vasinfectum Atk. v. *lutulatum* (Sherb.) Wr. (4494)	do	0	...
Roseum			
F. herbarum (Cda.) Fr. f. *1* Wr. (4220)	Sweden	0	...
Do. f. *2* Wr. (2826)	Germany	0	...
Do. v. *avenaceum* (Fr.) Wr. (4254)	Sweden	0	...
Gibbosum			
F. scirpi Lamb. et Fautr. v. *acuminatum* (Ell. et Ev.) Wr. (1725)	Germany	0	...
F. equiseti (Cda.) Sacc. (3250)	do	0	...
Do. (4229)	Sweden	0	0
Discolor			
F. culmorum (W. G. Sm.) Sacc. (4420)	Germany	0	...
Lateritium			
F. lateritium Nees v. *fructigenum* (Fr.) Wr. (4421)	do	+	+
Martiella			
F. solani (Mart.) v. *martii* (App. et Wr.) Wr. (4230)	Sweden	0	...
UNIDENTIFIED			
Fusarium sp. (J. II)	Japan	0	...
Do. (J. III)	do	0	...

[a] + indicates that the fungus strain was pathogenic; 0, nonpathogenic; ..., not tested.

[b] The culture numbers used in all tables are those of H. W. Wollenweber for the European forms, those of T. Hemmi for the Japanese forms designated by Roman numerals, and those of Wisconsin for 305, 435, and J. A.

pathogenicity. As evidence of this, *F. conglutinans* v. *callistephi* and *F. conglutinans* v. *majus* were pathogenic to China aster, while *F. conglutinans* and *F. conglutinans* v. *betae* were not. Likewise, *F. oxysporum* from cabbage, potato, and beet did not induce wilt of aster, while 5 strains of *F. oxysporum* f. *6*, isolated from diseased asters, proved pathogenic. These results, therefore, so far as they go, are in accord with the concept of narrowly limited host specialization in the parasitism of Fusaria of the section *Elegans*.

RESISTANCE TO VARIOUS FUSARIA OF STRAINS OF CHINA ASTER SUSCEPTIBLE AND WILT-RESISTANT WITH RESPECT TO F. CONGLUTINANS V. CALLISTEPHI

Representatives of the aster strains, which had been previously selected for wilt resistance to both the Beach and the Jackson isolations of *Fusarium conglutinans* v. *callistephi* (13), were tested for wilt resistance toward each of the other Fusaria found pathogenic to aster in the present studies. In the following discussion the terms susceptible and resistant refer to the behavior of the aster strains with respect to the above isolations of *F. conglutinans* v. *callistephi*. Trials with the entire series of pathogenic Fusaria were made in the greenhouse and with certain of the forms in out-door cloth-covered enclosures.

Greenhouse Trials

The following procedure was used for the greenhouse studies. Seedlings of an aster strain susceptible to *Fusarium conglutinans* v. *callistephi*, and of 3 strains resistant to this organism were grown in flats of wilt-free soil. When the second leaf appeared the plants were transplanted to flats, 8 rows of 6 plants each, 2 rows to an aster strain. Thus each flat contained 12 susceptible and 36 resistant plants. Inoculations were made at the time of planting as described in an earlier section. Three series of trials were run, a total of 36 susceptible and 108 resistant plants being tested with each organism.

The results of the greenhouse trials are summarized in table 2. To all of the Fusaria pathogenic to aster that were used in these trials, with the exception of the Japanese strain of *F. oxysporum* f. *6* (J. I), the susceptible strain of aster was relatively susceptible, while the 3 wilt-resistant strains proved relatively resistant. Figure 1 is representative of the results obtained. In other words, with one exception, these various Fusarium strains behaved similarly in respect to pathogenicity to the isolations of *F. conglutinans* v. *callistephi* used. Another exception will be mentioned later in this paper. It should be noted that the Fusaria thus agreeing closely in their pathogenicity and inducing essentially similar wilt diseases represent different varieties, species, and even sections as regards their morphological

TABLE 2.—*Comparative reaction to various Fusaria of susceptible and wilt-resistant strains of China aster. Greenhouse trials*

Fusaria Species and culture number	Susceptible asters	Resistant asters
	Per cent resistant	Per cent resistant
F. conglutinans v. *callistephi* (305)	14	94
Do. (435)	8	88
Do. (1472)	14	97
Do. v. *majus* (3206)	14	97
Do. (4422)	47	95
Do. (J. A)	50	92
Do. (J. VI)	25	83
F. oxysporum f. *6* (4179)	28	85
Do. (4200)	17	93
Do. (4497)	28	98
Do. (4506)	25	93
Do. (J. I)	58	58
F. lateritium v. *fructigenum* (4221)	25	93

and cultural characters. On the other hand, in the same "forma" (*F. oxysporum* f. *6*) different isolations behaved differently toward the wilt-resistant asters. Thus the morphological and cultural characteristics were not an index of pathogenicity.

Out-door Cage Trials

Out-door cloth-covered-enclosure tests with certain of the pathogenic Fusarium strains were made in the summers of 1931 and 1932. Seedlings of the aster strains to be tested were grown in flats of wilt-free soil. When the second leaf appeared they were transplanted to other flats of wilt-free soil. When the plants were 9 weeks old, they were planted in the field. Inoculations were made at this time. The inoculum had been prepared by transferring the organism to quart jars half-full of a sterile mixture of corn meal and sand. These were incubated at room temperature for 15 days. Then the inoculated corn meal and sand were mixed with wilt-free soil in the proportions of 1 to 5 and the whole incubated at room temperature for 7 days. At the time of planting approximately 100 grams of this inoculated soil was placed around each plant. In 1931 the asters were grown in ground where no asters had previously been planted, using 10 susceptible plants and 30 resistant plants representing 3 strains, with each organism. In 1932 the asters were grown in plots of steamed soil, 30 susceptible plants representing 2 strains, and 60 resistant plants representing 3 strains, being tested with each Fusarium. The necessity for growing the

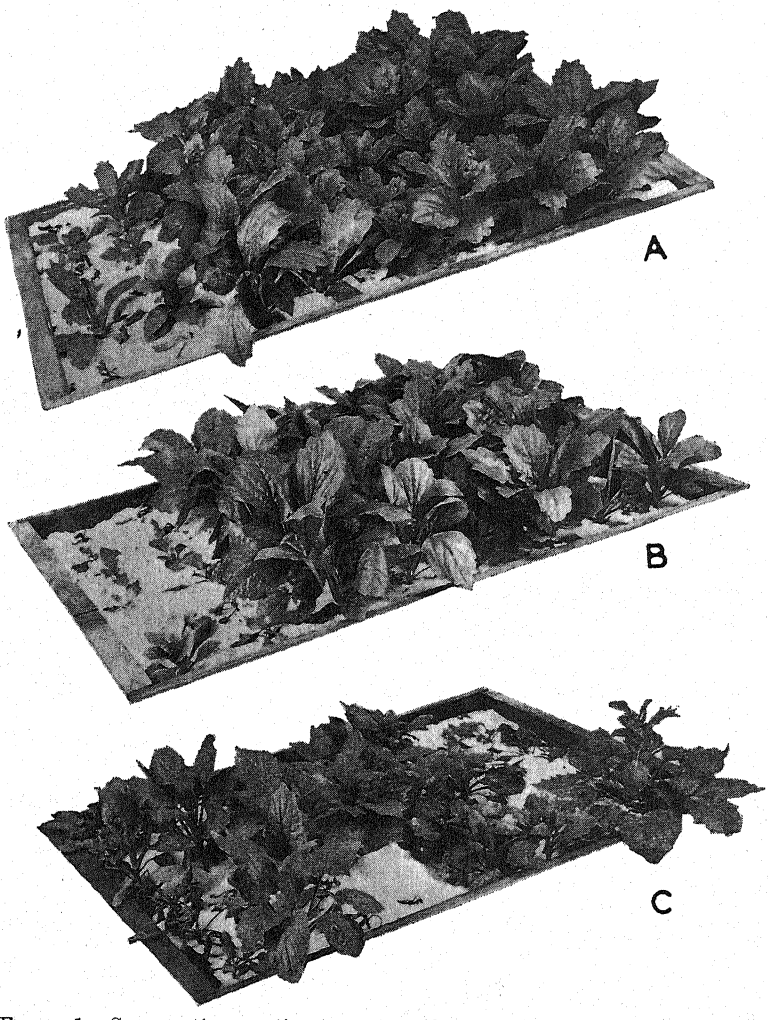


FIGURE 1. Comparative reaction to various Fusaria of susceptible and wilt-resistant strains of China aster. Greenhouse tests in shallow boxes or "flats."

The soil in each box was inoculated with a different Fusarium, and planted alike with 8 rows of asters, 6 plants to each row. The 2 rows at left in each box were susceptible to the Beach and the Jackson isolations of *F. conglutinans* v. *callistephi* and the 6 rows at right were resistant, including 2 rows each of 3 different aster strains.

The organisms used in the soil inoculation were:

A. *F. conglutinans* v. *callistephi* (305). This is the Beach isolation of the aster-wilt organism. Note the general sickness of the susceptible asters, 2 rows at left, and contrasting vigor of the resistant asters at right. The behavior of this organism is also featured in plate I, C.

B. *F. conglutinans* v. *majus* (3206). This isolation from Germany has, in all Wisconsin tests, shown essentially like pathogenicity with the Beach isolation of *F. conglutinans* v. *callistephi* (Figure 1, A; Plate I, C). The behavior of this organism is also featured in plate I, C.

C. *F. oxysporum f. 6* (J. I). This strain from Japan is quite as pathogenic to the resistant asters, at right, as to the susceptible ones at left.

Before photographing, the soil was covered with white sand in order to secure clearer differentiation of the sickly plants.

asters under cloth (13) so limited the available space as to preclude using larger numbers in the trials and employing the entire series of pathogenic Fusaria in these studies.

The results are shown in table 3 and plate I. The behavior of the various Fusaria during the two seasons is essentially similar and agrees

TABLE 3.—*Comparative reaction to various Fusaria of susceptible and wilt-resistant strains of China aster. Out-door cage trials*

Fusaria Species and culture number	Susceptible asters	Resistant asters
	Per cent resistant	Per cent resistant
SEASON OF 1931		
F. conglutinans v. *callistephi* (305)	30	93
Do. v. *majus* (3206)	30	100
F. oxysporum f. *6* (4200)	10	87
F. lateritium v. *fructigenum* (4221)	20	93
SEASON OF 1932		
F. conglutinans v. *callistephi* (305)	0	92
Do. v. *majus* (3206)	2	93
Do. (J. A)	0	92
Do. (J. VI)	2	97
F. oxysporum f. *6* (4200)	0	93
F. lateritium v. *fructigenum* (4221)	8	97
Noninoculated	93	98
Do.	88	98

with that in the greenhouse trials. Since the Japanese strain of *Fusarium oxysporum* f. *6* was not used in the out-door cage tests, the susceptible aster strains were relatively susceptible to all the pathogens, while the resistant strains proved relatively resistant to all.

Results of Collaborators

During the past few years the wilt-resistant aster strains developed at Wisconsin have been tested abroad as well as in various localities in this country. Several references to the behavior of these strains outside the United States have appeared in the literature. MacLeod (15) in New Brunswick, Canada, using 77 strains of aster, obtained 7 to 100 per cent infection in soil infested with *Fusarium conglutinans* v. *callistephi,* except with 5 Wisconsin strains that proved completely resistant to wilt on the soil used. Ogilvie and Mulligan (19), in England, tested 5 Wisconsin varieties, other wilt-resistant American varieties, and the common English market varieties on soil infected with *F. conglutinans* v. *majus.* The English market varieties gave almost 100 per cent wilt. On the other hand, the

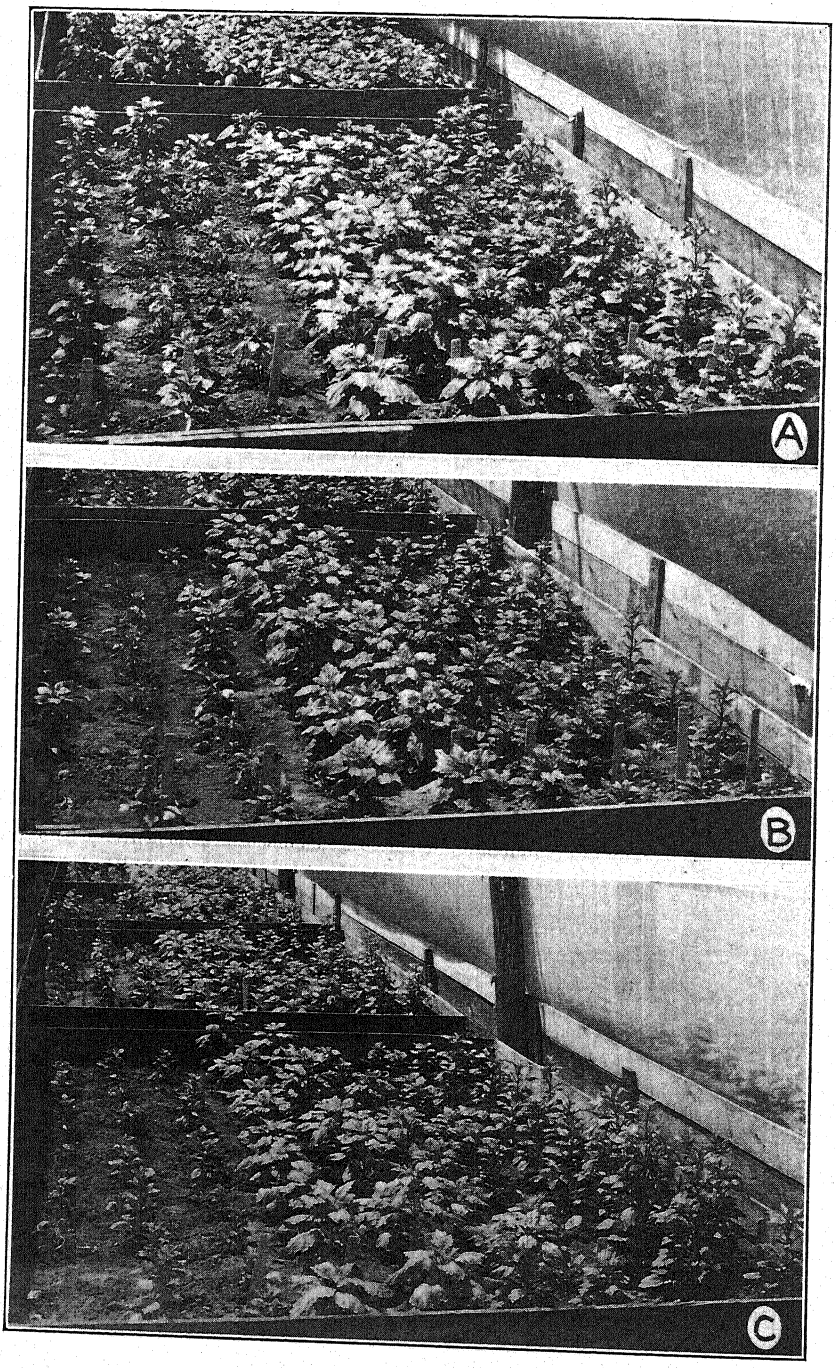


PLATE I

Wisconsin aster strains showed no wilt and the other American resistant varieties averaged 10 per cent wilt. On soil infected with *F. conglutinans* v. *callistephi,* where a planting of Giant Comet White had been severely attacked, 4 Wisconsin varieties showed almost no wilt. Wager (36), in South Africa, where the wilt is caused by *F. conglutinans* v. *majus,* found the 7 Wisconsin varieties moderately resistant, since an average of 47 per cent wilt was obtained, whereas the control variety showed 99 per cent.

These reports are in agreement with the results detailed in this paper that the Wisconsin wilt-resistant aster strains may prove more resistant than commercial susceptible strains[5] not only toward *F. conglutinans* v. *callistephi,* but also toward *F. conglutinans* v. *majus.*

Indiana Situation

While, in general, reports regarding the selected Wisconsin aster strains were favorable as to their resistance to fusarium wilt, one notable exception occurred in this country. E. R. Honeywell, in trials at the Purdue University (Indiana) Experiment Station, found supposedly resistant strains

[5] There are now on the market a number of commercial wilt-resistant strains that have been developed by selection from the Wisconsin or other aster strains largely by George J. Ball, West Chicago, Illinois, and Bodger Seeds, Ltd., El Monte, California.

PLATE I. Comparative reaction to various Fusaria of susceptible and wilt-resistant strains of China aster. Out-door cage trials 1932.

The steamed soil in each compartment, except the control, was inoculated with a different isolation of Fusarium, and planted alike with 9 rows of asters, 10 plants to each row. The 3 rows at left in each compartment were susceptible to the Beach and the Jackson isolations of *F. conglutinans* v. *callistephi* and the 6 rows at right were resistant, including 2 rows each of 3 different aster strains.

The organisms used in the soil inoculation were:

A. *F. lateritium* v. *fructigenum* (4221) from Germany in front plot. A little of the noninoculated control compartment shows in the background.

B. *F. oxysporum* f. *6* (4200) from Germany in front plot. The pathogenicity is essentially like that of the Beach isolation of *F. conglutinans* v. *callistephi* shown in figure 1, A and plate I, C, and in marked contrast with the Japanese isolation of *F. oxysporum* f. *6* (J. I) shown in figure 1, C. In the back plot *F. lateritium* v. *fructigenum* (4221) was used (Plate I, A).

C. In plots from front to back:

F. conglutinans v. *callistephi* (305) from Michigan. This is the Beach isolation and its behavior is also featured in figure 1, A.

F. conglutinans v. *majus* (3206) from Germany. The behavior of this organism also is featured in figure 1, B.

F. oxysporum f. *6* (4200) from Germany (Plate I, B).

F. lateritium v. *fructigenum* (4221) from Germany (Plate I, A).

Note that in all cases the organisms showed essentially like pathogenicity. The susceptible asters to the left were generally sick, while the resistant asters to the right were almost all healthy.

extremely susceptible to wilt. Brief discussions of his results have appeared (27, 28, 29, and 10). Riker saw Honeywell's trials in Indiana in the summer of 1932. Subsequent greenhouse tests at Wisconsin confirmed the Indiana results. In these, two methods were employed (1) using aster-sick Indiana soil; (2) using soil inoculated with organisms isolated from the Indiana soil.[6] In both cases the results were the same. The Indiana fungus was fully as pathogenic with the resistant Wisconsin aster strains as with the susceptible aster strains. This exceptional Indiana pathogen was sent to H. W. Wollenweber, who identified it as *Fusarium conglutinans* v. *majus*. It is to be noted that isolations of *F. conglutinans* v. *majus* from Germany and Japan in trials reported in this paper, and from England (19), and South Africa (36) in other experiments, have in general agreed in pathogenic behavior with the typical aster-wilt organism, *F. conglutinans* v. *callistephi*. As mentioned earlier, one of the Japanese isolations likewise proved pathogenic to the Wisconsin wilt-resistant aster strains. It was, however, rather less virulent than the Indiana fungus. This Japanese fungus has been identified by H. W. Wollenweber as *F. oxysporum* f. *6*. The outcome of a trial with this organism is illustrated in figure 1, C. It is noteworthy that here, again, is evidence that like types of pathogenicity may occur with Fusaria differing in morphological or cultural characters.

While these results are limited in extent, they are fully convincing that aster wilt Fusaria occur to which the Wisconsin selected aster strains are highly susceptible. Experience with the vascular Fusaria, in general, would encourage the expectation that strains of aster may be found adequately resistant to this type of pathogen. Wisconsin conditions have not permitted continued search along this line. This can best be undertaken wherever the pathogen is detected locally, as in Indiana or Japan. The reports from Indiana, cited above, show that progress already is being made by E. R. Honeywell in this direction.

DISCUSSION

The experiments outlined in this paper indicate that morphological and cultural characters and pathogenicity among the Fusaria are not necessarily interrelated. Evidence for this was obtained in several ways. Fusaria closely related according to classification differed in their ability to attack the China aster. For example, *Fusarium conglutinans* v. *callistephi* and *F. conglutinans* v. *majus* were pathogenic to aster while *F. conglutinans* and *F. conglutinans* v. *betae* were not. Likewise, different isola-

[6] The writers wish to express appreciation of the kind assistance both of E. R. Honeywell who furnished samples of aster-sick soil from which isolations were made at Wisconsin, and of R. W. Samson, who supplied cultures of organisms isolated from diseased asters at Lafayette, Indiana.

tions of the same fungus differed in relative pathogenicity. An instance of this was *F. conglutinans* v. *majus*. The Wisconsin resistant asters were relatively resistant to isolations of this fungus from Germany and Japan, but extremely susceptible to an isolation from Indiana. On the other hand, Fusaria classified as of different varieties, species, and even sections behaved similarly as to pathogenicity. Most isolations of *F. conglutinans* v. *majus*, *F. oxysporum* f. *6*, and *F. lateritium* v. *fructigenum* used, agreed with the Beach isolation of *F. conglutinans* v. *callistephi* in that the Wisconsin resistant asters proved relatively resistant and the susceptible asters relatively susceptible.

SUMMARY

1. Pathogenicity tests with 10 Fusaria of the section *Elegans* that induce wilts on other hosts gave negative results with China aster.

2. Pathogenicity tests with 27 strains of Fusaria isolated from wilting asters showed that: (a) Some of the Fusaria were pathogenic to aster while some were not. (b) Of the pathogenic forms, most belonged to the section *Elegans*, but *Fusarium lateritium* v. *fructigenum* did not. (c) Not all of the Fusaria of the section *Elegans* isolated from diseased asters were pathogenic to aster.

3. Fusaria closely related morphologically and culturally, *i.e.*, merely varieties of the same species, differed in their ability to induce wilt of aster.

4. Several Fusaria (*F. conglutinans* v. *majus*, *F. oxysporum* f. *6*, and *F. lateritium* v. *fructigenum*), pathogenic to aster, but, according to standard methods, classified as a different variety or species or even in a different section, induced a wilt of aster essentially similar to that caused by the type strain of *F. conglutinans* v. *callistephi*. This was true for most of the isolations of these Fusaria tested not only as to general symptoms, but also as to relative pathogenicity. Asters both susceptible and wilt-resistant to the Beach and the Jackson isolations of *F. conglutinans* v. *callistephi*, proved, respectively, susceptible and wilt-resistant to a number of these other Fusaria.

5. Two exceptions were found to this agreement as to relative pathogenicity. One of these from Indiana was identified by H. W. Wollenweber as *F. conglutinans* v. *majus*, the other from Japan as *F. oxysporum* f. *6*. These 2 isolations caused as much wilt among the aster strains resistant to the Beach and the Jackson isolations of *F. conglutinans* v. *callistephi* as among those susceptible. Isolations of each of these Fusaria from other sources behaved essentially like the Beach isolation of *F. conglutinans* v. *callistephi*.

6. Thus the evidence seems to justify the conclusion that (a) the morphological and cultural characters of the Fusaria associated with aster wilt

are not a reliable index of pathogenicity, and (b) that essentially the same type of aster wilt may result from the attack of Fusaria classified as of different varieties, or species, or even sections.

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RHIZOCTONOSIS OF POTATOES GROWN UNDER IRRIGATION

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INTRODUCTION

The rhizoctonia disease of potatoes, caused by *Corticium vagum* Berk. and Curt., is still of major importance in the potato-growing areas of Colorado. Both stem and tuber infection are important phases of the disease. Stem infection includes the underground area of the plant. Under favorable conditions infection may involve the whole root system. Under normal growing conditions it is not serious in the expression of symptoms, but the loss due to reduced vitality of the plant and subsequent decrease in yield often is underestimated.

In the warmer soils of the irrigated areas, stem infection is seldom a limiting factor in potato culture. This type of infection often causes serious losses in the early crop and in those grown at high altitudes in the mountain valleys, where soil temperatures remain relatively low throughout the growing season.

In the irrigated areas of Colorado a wide variation of physical factors exists. The large area located east of the mountain range extending from north to south through the state is commonly called the eastern slope or the plains area. Certain sections close to this mountain range exist as cultivated farms only through the aid of irrigation water. Summer temperatures are usually high during the day but comparatively low at night. Two crops of potatoes, with reference to time of planting, are grown. The early crop consists of the varieties Bliss Triumph, Irish Cobbler, and Early Ohio, which make up the bulk of the acreage, and is planted between April 1 and May 10. This early crop matures marketable potatoes from July 15 to August 15. The late or main crop consists chiefly of two varieties, Rural New Yorker and Bliss Triumph, and is planted between May 25 and June 15. This crop is harvested from September 15 to October 30.

Varieties and planting dates in the mountain valleys vary with the location of the area in which the potatoes are grown. Many of the higher valleys are not free from frost until June 1. The soil usually remains cool and wet throughout the growing season and it is here that Rhizoctonia does most damage to the underground portions of the potato stems.

Observations and experiments have shown that stems of the early-crop potatoes often become infected with Rhizoctonia. In many cases the young sprouts are killed by the fungus before they reach the surface of the soil. Very little stem infection is found on the late- or main-crop potatoes of the

Greeley, Colorado, area. The soil temperature is quite high by June 1, and rapid germination follows planting of the seed. Tuber infection is rarely a factor on the early crop. Tubers harvested in September often show heavy infection.

With these facts in mind the writer undertook a series of experiments designed to determine the relation of temperature and moisture to infection of potatoes by Rhizoctonia. The experiments were undertaken with the hope of obtaining information that would be of value in formulating certain control measures based on cultural practice along with certain seed treatments.

REVIEW OF LITERATURE

A survey of the literature reveals the fact that rhizoctonosis is one of the most common diseases affecting the potato. It has been reported as damaging potatoes throughout the various potato-growing areas of the United States and Alaska. Alberts (1) reported rhizoctonosis as one of the most serious diseases of the potato in Alaska. Gratz (7) reported it from Florida. Many investigators have reported the disease on stems and tubers throughout the more temperate zone.

The influence of such factors as temperature, soil moisture, and strains of the fungus have not been definitely settled. All investigators seem to agree that conditions favorable for stem infection differ in some respects from the optimum for the development of sclerotia on the tubers. Gratz (7) found that stem lesions were quite common in Maine but seldom a factor in Florida. Richards (15), reporting on his studies in Wisconsin, says "The results in general indicate that the occurrence of an average daily temperature of 21° C.—on the growing points during the first six weeks of growth may accurately govern the damage done to the crop by the fungus in any one season. Richards (14) again found that the fungus may be parasitic through a wide range of temperatures and that serious damage to growing point and tissue occurred at 18° C.

Edson and Shapovalov (6) found that several species of Rhizoctonia are capable of attacking the underground portions of the potato stem. Rolfs (16) concluded that a high temperature and abundant moisture were essential for the rapid development of the fungus. He correlates the seriousness of the disease in Colorado with the practice of excessive irrigation during periods of high soil temperature. Peltier (12) concluded that a high temperature, 88° F., and moisture content of the soil above or below that most favorable for plant development, determined to a large degree, the virulence of strains. He evidently referred to air temperature, since no data on soil temperature were given with reference to the above conclusion. Balls (2), working in Egypt, found that low temperature favored the devel-

opment of the rhizoctonia fungus on cotton and that in some cases the plant might not become infected even when growing in a soil heavily infested with the active mycelium.

The greatest amount of work has been done on the stem-lesion phase of the disease. The sclerotial stage found on the tubers, apparently, has been considered less important than the stem-infection injury, and several investigators have studied the factors influencing that type of injury. Duggar (5) reported 2 types of infection, the common sclerotial type and the scab type. Rolfs (17) described the cracked, scabby type as damaging potato tubers in Colorado. Matsumoto (10) discussed strains of the rhizoctonia organism on many hosts. He reported 6 different strains and that all showed increased growth on the acid side of neutrality. Ramsey (13) described a form of rhizoctonia injury resembling scab, in some respects. Rosenbaum and Shapovalov (18) showed quite conclusively that strains of the fungus exist and that certain strains are more pathogenic on potatoes than others.

Considerable work has been done on the control of rhizoctonosis of potatoes. Most of the control measures have been directed towards the killing of the tuber borne sclerotia. Schultz, Gratz, and Bonde (19) found that in Maine seed-potato treatment with most of the materials commonly used for that purpose reduced stem infection and tuber-borne sclerotia. They found that soaking tubers in a solution of corrosive sublimate was the most effective treatment. Clayton (3), working in the Long Island area of New York State, found that organic mercury compounds were less effective against Rhizoctonia than was corrosive sublimate. He also concluded that seed treatment was not profitable for the Long Island grower, since soil infection was high. MacMillan and Christensen (9) found mercuric chloride effective in the control of rhizoctonosis in the form of a commercial gain as expressed in yield. McKay (8) found that the 1½-hour mercuric chloride treatment was superior to the half-hour treatment and that formaldehyde was ineffective. Stakman and Tolaas (20) found seed selection and seed treatment with corrosive sublimate and formaldehyde effective against Rhizoctonia. Their results showed corrosive sublimate more effective than formaldehyde. Melhus and Gilman (11) described in detail the hot-formaldehyde method of treating seed potatoes for control of rhizoctonosis and scab. This method is popular in the Greeley area. Dana (4), working in Washington, made a comprehensive study of Rhizoctonia. He took up several phases of the disease and recommended a combination of seed treatment and certain cultural practices for its most effective control.

GENERAL METHODS

The period covered by the studies here presented includes the growing seasons of 1930–31–32. The region covered includes several potato-growing

areas located in various sections of Colorado. Experimental data, for the most part, were taken from experiments conducted on the Colorado Potato Experiment Station at Greeley, Colorado. Observations relative to occurrence and severity of infection were made in the irrigated areas of both the plains and mountain sections. Control experiments included seed treatment and cultural practices as affecting the sclerotial stage of the disease on the tubers. The variety Bliss Triumph was used in all of the experiments.

The following experiments, for the most part, were conducted in the field. Laboratory and greenhouse work supplemented the field experiments. Soil temperatures were recorded by means of soil thermographs with the bulb placed at a depth of 5 inches. Soil moisture content was not determined. Controlled irrigation served to vary the amount of moisture received by each experimental plot. A plot, designated as heavy irrigation, received water in excess of normal plant needs. Another plot received light irrigation, which consisted of frequent applications of water but in quantities less than was necessary for maintenance of a vigorous growth of the plants.

Several types of seed tubers were used in the field experiments. Infected tubers consisted of those having a large number of rhizoctonia sclerotia. Clean tubers were free from any appreciable amount of sclerotial infection. In some cases the infected tubers were planted without treatment to insure the presence of inoculum. In others the infected seed tubers were treated, the object being to test the efficiency of several seed disinfectants. Tubers were considered free from the fungus when no sclerotia were visible to the naked eye. In several experiments this type of tuber was treated to insure complete freedom from the fungus.

In estimating the amount of disease present on stems, each stem was cultured and the organism identified. The amount of infection on tubers was determined by careful examination of each one from an experimental unit.

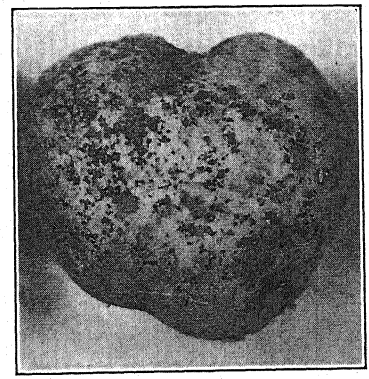


FIG. 1. Sclerotia on tuber. Medium heavy infection.

Those showing an appreciable number of sclerotia were considered infected. Figure 1 shows typical medium heavy sclerotial tuber infection.

RELATION OF SOIL TEMPERATURE TO STEM INFECTION

The yield of early potatoes in the Greeley, Colorado, section, usually is lower than that of those planted as a late or main crop. A higher market price usually compensates for the smaller yield; consequently, growers plant as early as possible. Many fields are planted during the first week of April, so these young plants often are injured by frost.

Stem lesions caused by Rhizoctonia (*Corticium vagum*) often do considerable damage to this early crop. Low soil temperature and rhizoctonia damage on the stems appear to be closely related. In the higher altitude of the mountain valleys, where low soil temperatures continue through most of the growing season, stem lesions are considered as one of the limiting factors to potato culture. Ninety per cent infection has been noted in several mountain valleys and also in the early potato section near Greeley. The stems often become infected before the young shoots are above ground, and this girdling often kills the sprouts and causes poor stands. The more common type of injury is the lesion infection, where only a part of the stem is injured. The plant does not die out but its vitality is reduced. As the season progresses and the soil becomes warmer these plants often recover and produce a fair yield.

An experiment designed to determine the effect of soil temperatures on rhizoctonia infection on the stems of potatoes, was conducted in the early crop section near Gilcrest, Colorado, in 1932. Sixty seed pieces bearing a large number of sclerotia were planted each week for six weeks, beginning April 1. Each week as the infected seed was planted, a check plot of the same number of hills was planted with seed free from sclerotia. Soil-temperature data were taken at a depth of 5 inches. Accurate soil-moisture records were not taken.

Six weeks after planting each lot was dug and the stems were examined for rhizoctonia infection. Those stems showing infection were cultured, the organism isolated and identified. Table 1 shows the results of this experiment. Data from the check plots were not included in the table, since the amount of infection was light and not uniform. The greatest amount of infection occurred on the plot planted April 8. Of the 60 stems examined, 44 showed rhizoctonia infection. Of these, 31 contained lesions covering large areas of the stem. The percentage of infection was high on the plot planted April 1, but the type of injury was less severe than on the plot planted April 8. Tubers planted after May 1, became only slightly infected. Only 8 of the 60 plants showed infection and none showed presence of lesions covering a large area of the stem. Figure 2 shows stem with typical lesions.

TABLE 1.—*Effect of soil temperature on infection of potato stems by Rhizoctonia. Records made 44 days after planting. Greeley, Colorado, 1932*

Number of plants	Date planted	Date stems were examined	Number of stems showing infection	Number of stems showing severe infection	Mean min. soil temperature 44 day period	Mean maximum temperature 44 day period	Mean temperature 44 day period
					°*F.*	°*F.*	°*F.*
60	April 1	May 15	32	16	39.5	61.0	50.2
60	April 8	May 22	44	31	41.8	63.5	52.6
60	April 15	May 29	37	23	44.3	65.7	55.0
60	April 22	June 5	26	2	45.4	67.5	56.4
60	April 29	June 12	20	4	48.2	72.3	60.2
60	May 7	June 20	8	0	55.0	73.5	64.3

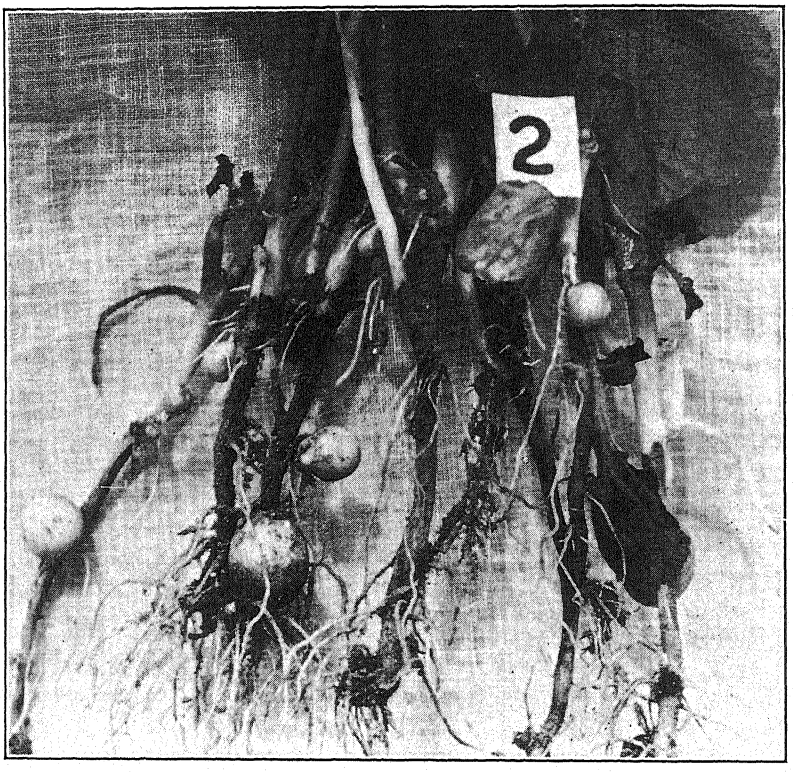


FIG. 2. Typical stem lesions caused by Rhizoctonia.

The average minimum soil temperature for the period April 8 to May 22 was 41° F., and the average maximum was 63.5° with a mean of 52.6° for the 44-day period. The most severe infection occurred during this 44-day period, indicating that the optimum soil temperature for stem infection is between 41.8° F. and 63.5°. These results agree with field observations that the most severe stem infection occurs early in the season when soil temperatures are quite low. Although the fungus is parasitic throughout a wide range of temperatures, as stated by Richards (15), the lower soil temperatures of the early growing season and the higher mountain valleys no doubt favor the development of the stem-lesion phase of the disease.

THE EFFECT OF LIGHT, NORMAL, AND HEAVY IRRIGATION ON THE DEVELOPMENT OF SCLEROTIA ON TUBERS

Preliminary experiments and observations made in several commercial fields indicated that moisture in excess of the normal needs of potato plants

is an important factor in the development of the sclerotial stage of *Corticium vagum*. When potatoes were given an excess of moisture throughout the growing season, many of the tubers were found to be heavily infected with sclerotia. An experiment to test the effect of excess irrigation water on the infection and development of sclerotia was started during the season of 1931. Both clean and infected tubers were used. The amount of irrigation water was varied by the number and amount of applications each lot received. Temperature data were recorded by soil thermographs with the bulbs placed at a depth of five inches. Each plot consisted of a row containing $\frac{1}{16}$ acre. All tubers from a plot were examined and the percentage of infected ones computed on the basis of the total weight of tubers from the plot.

In table 2, are shown the kind of seed tubers used, the amount of water applied, the percentage of infected tubers harvested, and the soil temperature for the different plots. Tubers containing sclerotia were used in several of the units to insure the presence of inoculum. The rows receiving heavy irrigation were watered as often as was necessary to keep the soil nearly saturated. The units receiving normal irrigation were given sufficient water to keep the plants in an active growing state. Light irrigation consisted of fewer light applications of water than was considered good practice. Plots with no irrigation received only such water as fell in the form or rain, which was not of sufficient quantity to allow normal plant growth.

The degree of infection in the different plots varied considerably. Any tuber having on it sufficient sclerotia to be readily visible was considered infected. Many of the tubers were so heavily infected as to be discriminated against by buyers.

The highest percentage of rhizoctonia infection was found on the tubers from the plot receiving heavy irrigation. The season's mean soil temperature was lowest in this plot. Table 2, shows the soil-temperature data taken in the plots receiving light, normal, and heavy irrigation. The percentage of tuber infection corresponded closely with the amounts of water applied and the correspondingly low mean soil temperature. That the soil temperatures varied but little between the three plots representing the different types of irrigation, leads to the conclusion that *Corticium vagum* is less sensitive to temperature variations than to the moisture content of the soil.

The presence of inoculum on the seed pieces increased the amount of infection on the plots receiving heavy and normal irrigation. There was but little increase from this cause in the plots grown under light or no irrigation. The results, in a measure, agree with those obtained by Rolfs (16) who found that abundant moisture and high temperature favored the

TABLE 2.—*Relation of irrigation to the development of sclerotia on tubers. Greeley, Colorado. 1931*

Condition of seed	Type of irrigation	Total weight of tubers	Infection	Mean max. soil temp.	Mean min. soil temp.	Season mean soil temp.
		Pounds	*per cent*	°*F.*	°*F.*	°*F.*
Infected	Heavy	229	30.4	82.1	65.7	73.9
Infected	Normal	308	9.5	79.4	68.1	73.7
Infected	Light	226	3.0	82.6	67.7	75.1
Infected	None	93	3.0	No data	No data	No data
Clean[a]	Heavy	310	13.0	" "	" "	" "
Clean	Normal	306	3.5	" "	" "	" "
Clean	Light	197	3.0	" "	" "	" "
Clean	None	83	2.5	" "	" "	" "

[a] Seed treated with mercuric chloride, 1–1000, 1½ hours.

development of the fungus. Especially did he find that abundant moisture is essential for optimum development of sclerotia.

EFFECT OF HEAVY IRRIGATION NEAR THE END OF THE GROWING SEASON ON THE DEVELOPMENT OF SCLEROTIA ON TUBERS

Observations in rhizoctonia-infested fields have shown that sclerotial infection on tubers is always most serious in plots that had received large quantities of irrigation water during the last 30 days of the growing period. This practice of heavy, late irrigation is common in the Greeley area.

An experiment to determine the effect of heavy, late-season irrigation on the development of sclerotia on tubers was started in the season of 1931 and carried through that of 1932. Both infected and clean tubers were planted in 2 plots of 4 rows each. Each row contained $\frac{1}{16}$ A. Both plots received normal irrigation until approximately 30 days before harvest, when the heavy-irrigation plot was given several copious applications of water. Only light applications were given to the normally irrigated plot. In table 3 the results of this experiment for the 1931 season are given. Those of the 1932 season were essentially the same and are not given.

The tubers from the heavily irrigated plot were covered with soil that was removed with difficulty, because of large numbers of sclerotia. Those from the normally irrigated plot were clean and essentially free from sclerotia. Each tuber from both plots was carefully examined for sclerotia and the percentage of infection computed on the basis of total weight of tubers.

The number of sclerotia on the tubers varied, but when present they were in sufficient numbers to be readily noticed. Many tubers from plot 2 were almost unsalable because of many sclerotia. The infected tubers produced tubers containing the highest percentage of sclerotial infection. Each plot planted with clean tubers produced cleaner ones. Yield was affected but little by the two types of irrigation, the normally irrigated plot produced a slightly higher yield.

There is but little difference in the mean soil temperature for the last 30 days growth period of the two plots. The lower mean temperature existed in the heavily irrigated plot, but is of such small difference that it seems doubtful if soil temperature was a factor in the results of the experiment.

SEED TREATMENT AS A CONTROL OF TUBER INFECTION

Several seed treatments have been recommended by different investigators for control of stem infection caused by *Corticium vagum*. Schultz *et al* (19) found that seed treatment with most of the materials commonly used reduced both stem and tuber infection. Clayton (3) found mercuric

TABLE 3.—*Effect of heavy late-season irrigation on the development of sclerotia on tubers. Greeley, Colo., 1931*

Row	Plot 1					Plot 2				
	Normal irrigation					Heavy late irrigation				
	Condition of seed	Number of hills	Yield in pounds	Percentage of tubers with sclerotia	Mean soil temp. last 30 days	Condition of seed	Number of hills	Yield in pounds	Percentage of tubers with sclerotia	Mean soil temp. last 30 days
					°F.					°F.
1	Clean	190	322	3.1	71.3	Clean	190	281	14.2	67.2
2	Infected	187	281	10.2	70.6	Infected	190	259	44.3	67.3
3	Clean	190	304	3.4	69.9	Clean	187	287	10.0	67.0
4	Infected	186	324	14.0	70.6	Infected	189	291	35.1	68.5

chloride superior to organic mercury compounds for control of rhizoctonosis. He found also that when soil infestation was high seed-tuber treatment was of little value in the Long Island, New York, area.

In Colorado nearly all potatoes are grown under irrigation, and often the tubers are heavily infected with Rhizoctonia. Much locally grown seed is used and the problem of tuber treatment becomes an important factor for the control of stem lesions in the early-crop area and for the reduction of tuber infection in the late-crop sections.

Mercuric chloride, formaldehyde, and several of the commercial organic mercury compounds are used as seed disinfectants. Often the treatment must serve to control both rhizoctonosis and common scab, and mercuric chloride is most commonly used.

An experiment designed to test the value of several organic mercury compounds, mercuric chloride, and formaldehyde for the control of sclerotia on tubers was started in the season of 1931. The following experiments were conducted in the late-crop plots, where stem infection seldom is a problem.

The organic mercury compounds used included several supplied by the Bayer Co. and were known only by numbers. No name was supplied with the materials. The dilutions used were suggested by the manufacturers. Seed designated as clean was apparently free from sclerotia, since none were visible to the naked eye.

Two types of mercuric chloride treatment were used, the common 1–1000 treatment for 1½ hours, and the acidulated mercuric chloride solution. In the latter 6 ounces of mercuric chloride were dissolved in 1¾ pints of 31 per cent hydrochloric acid and added to 22 gallons of water. The whole tuber was allowed to remain in this solution for 3 minutes, then drained and dried. In the hot-formaldehyde treatment the cut tuber was immersed for 2 minutes in a solution containing 1 pint of commercial formaldehyde in 15 gallons of water held at 122° F., then removed and allowed to dry. In table 4 are given the results obtained from the several types of seed treatment.

Where sclerotia were present on the seed, all treatments were of value. The highest yield was from apparently clean seed treated with mercuric chloride. Untreated sclerotia-bearing seed produced a crop 47 per cent of which was infected, the infection varying from severe to light. Many of the tubers were so badly affected with sclerotia as to be discriminated against by buyers. Untreated clean seed produced a crop relatively free from infection.

Hot formaldehyde proved to be of less value than mercury chloride for killing the sclerotia on the seed tubers as indicated by the relatively

TABLE 4.—*Results of seed-potato treatment for control of sclerotia on tubers. Greeley, Colo., 1932*

Condition of seed planted	Treatment	Yield bu. per acre	Percentage of tubers bearing sclerotia[a]
Clean[a]	Mercuric chloride 1–1000 1½ hrs.	329.00	3.6
Clean[b]	Untreated	318.00	9.4
Sclerotia on surface	Mercuric chloride 1–1000 1½ hrs.	301.00	7.1
Sclerotia on surface	Untreated	294.50	47.0
Sclerotia on surface	Acid mercuric chloride. 3 min.	270.00	17.0
Sclerotia on surface	Hot formaldehyde 2 min. 122° F.	284.00	13.3
Sclerotia on surface	Bayer organic mercury #694	321.00	14.0
Sclerotia on surface	Bayer organic mercury #738[c]	234.00	11.7
Sclerotia on surface	Bayer organic mercury #664	299.10	15.5
Sclerotia on surface	Semesan Bel.	286.50	16.1

[a] By weight.
[b] Apparently clean seed.
[c] Poor stand. Seed piece injury by treatment.

high percentage of infected tubers. The 1–1000 mercuric chloride treatment was more effective than the acid mercuric chloride.

The 4 organic mercury compounds did not control sclerotial infection as effectively as did mercuric chloride. Bayer No. 694, increased the yield over the mercuric chloride and formaldehyde treatments, but the percentage of infected tubers was higher. The increased yields in one of the organic mercury plots was partly due to a better stand.

These results would indicate light soil infection by *Corticium vagum*, and also that most of the infection on the tubers resulted from infected seed.

SUMMARY

Rhizoctonosis of potatoes, caused by *Corticium vagum*, is common in the irrigated areas of the Greeley, Colorado, section. The stem-infection phase

of the disease is most common on the early-crop potatoes grown in a cool soil. Damage to the plants through reduced vitality caused by stem lesions may be the common type of injury in the early-crop areas. Tuber infection is rarely a factor in the early crop.

Experiments conducted near Gilcrest, Colorado, in the early-crop area, showed serious stem infection when the crop was planted early in cold soils. Plantings made late in the season are relatively free from stem infection.

Excess moisture and low soil temperatures have been observed to be coincident with heavy sclerotial infection on tubers. Experiments indicate that more severe tuber infection occurs under conditions of heavy irrigation. Lowered soil temperature caused by excessive irrigation apparently is not so important as is moisture in the development of sclerotia on tubers.

Irrigation water, applied in large quantities late in the season, increased the number of sclerotia on tubers. Careful handling of irrigation water towards the close of the growing season is suggested as a control measure for tuber infection.

Seed-tuber treatment is of value in reducing the amount of rhizoctonia infection on tubers. Mercuric chloride was found to be of more value than formaldehyde or any of the organic mercury compounds.

A combination of seed treatment and careful handling of irrigation water during the last few weeks of tuber growth is suggested for the control of tuber infection by rhizoctonia.

GREELEY, COLORADO.

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THE NATURE OF INHIBITION BETWEEN CERTAIN FUNGI PARASITIC ON CITRUS[1]

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INTRODUCTION

The importance of investigations on mixed cultures of organisms was pointed out concisely by Fawcett (2) in his statement " . . . since Koch and others perfected the pure-culture methods . . . students have been thoroughly imbued with the idea that cultures must be pure for a single organism. . . . The insistence on purity of cultures was, indeed, a very important condition for preliminary work for the study of the part played by a single organism in the absence of all others. . . . We have now come to a stage requiring synthesis as well as analysis, integration as well as differentiation. . . . Nature does not work with pure cultures alone but most frequently with associations."

According to the literature, interesting behaviors of mixed cultures of organisms have been reported, based both on accidental observations and on purposely made investigations.

In general the studies of mixed cultures have shown two different types of behavior; in some cases the culturing of one organism in the presence of another has resulted in the production of reproductive bodies; in other cases the associated culture of organisms has led to a partial, strong, or mutual inhibition in the rate of growth and size of the colonies of the organisms involved. Striking cases also have been shown where the presence of another organism greatly accelerated the growth or action of another or where the mutual action was much increased.

In connection with the first type of behavior (stimulation to formation of reproductive bodies), Molliard (13); Heald and Pool (7); Sartory (16) and (17); and McCormick (11), have reported some interesting cases.

In 1924, Porter (14) made an extensive study of combinations of fungi and of fungi with bacteria, and, after observing their behavior in culture, described 5 different types of inhibition.

Machacek (12) in his studies of mixed cultures on fruits, found out that *Penicillium expansum* and *Cladosporium fulvum* inhibited completely the growth of *Sclerotinia fructicola* and *Botrytis* sp., respectively.

Fawcett and Lee (4, p. 37–38) report that combined inoculations with *Phytophthora citrophthora* and *Fusarium* sp. on citrus trees, produced

[1] From a thesis submitted in partial fulfillment of the requirements for the degree of Master of Science, published with the approval of the Dean of the Graduate School, Pennsylvania State College.

larger lesions and the decay proceeded faster and became more severe than inoculations with *P. citrophthora* alone.

Savastano and Fawcett (18) conducted a series of experiments with mixed cultures of fungi on fruits, studying especially the effect of temperature on the behavior of the combinations and they found that temperature had a decidedly selective effect in many cases, enabling one organism in a mixture to dominate the others in the production of decay.

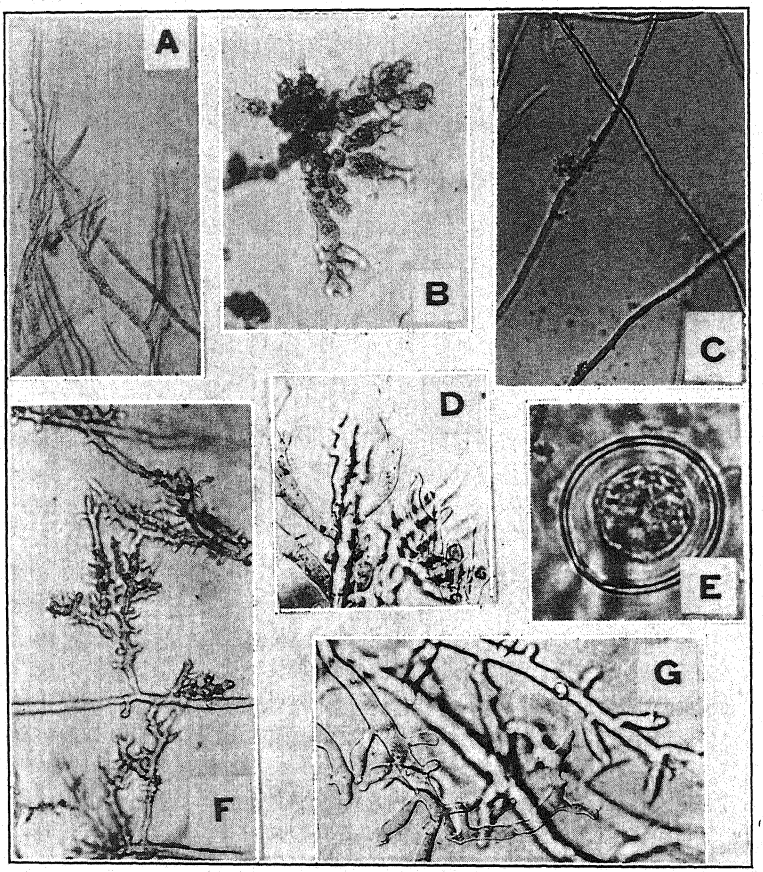


FIG. 1. A. Normal mycelium of *P. parasitica* when growing alone on potato-dextrose agar. B. Mycelium from the edge of a colony of *P. parasitica* growing within 3 mm. of a colony of *Diaporthe citri*, showing abnormal branching. C. Normal mycelium of *P. citrophthora* when growing alone on potato-dextrose agar. D. Effects of the same filtrate sterilized in the autoclave for 20 minutes at 20 lbs. pressure and 110° C., on *P. citrophthora*. E. Oogonia-like body of *P. citrophthora* as observed in liquid cultures and oranges. F. Mycelium from the edge of a colony of *P. citrophthora* growing within 3 mm. of a colony of *Diaporthe citri*, showing abnormal branching. G. Effect of the filtrate obtained from a 20-day-old culture of *Diaporthe citri* in Czapek's liquid media, on the mycelium of *P. citrophthora*.

Vasudeva (21) observed that the presence of *Botrytis allii* in the inoculum of *Monilia fructigena* reduced strikingly the pathogenicity of the latter.

Gioelli (5) reported that the presence of *Penicillium digitatum* completely checked the growth of *P. italicum* in fruit rots.

Reid (15) did some work on the bacterial inhibitory properties of the mold, *Penicillium notatum,* especially upon the nature of the filtrate containing the staling products of the mold.

Concerning the substance responsible for stimulation or inhibition in such cases little is definitely known. Some believe the action purely enzymatic, or a question of availability of nutrients; others think it due to an osmotic relationship or merely a matter of toxicity.

With these views in mind, the writer conducted a series of experiments with mixed cultures of fungi in an endeavor to throw more light on the subject.

MATERIALS AND METHODS

For the studies, 12 species of fungi, commonly associated with citrus fruits were used: *Penicillium digitatum* Sacc.: *P. italicum* Wehmer; *Colletotrichum gloeosporioides* Penz.; *Alternaria citri* Ellis and Pierce; *Diplodia natalensis* Evans; *Diaporthe citri* Wolf; *Mucor spinescens* Lendn.; *Sclerotium rolfsii* Sacc.; *Botrytis cinerea* Pers.; *Sclerotinia libertiana* Fckl.; *Phytophthora parasitica* Dastur; and *P. citrophthora* (Sm. & Sm.) Leonian.

The 12 fungi were first grown in Petri dishes in pairs. In these tests, all possible combinations of two were set up. Potato dextrose agar was used and inoculations were made with bits of mycelium from pure cultures, the two fungi being placed about 3 cm. apart. Similar combinations were set up on potato-dextrose agar in van Tieghem cells for more careful observation.

Of the combinations tried, the most interesting were: *Diaporthe citri* × *Phytophthora parasitica* and *D. citri* × *P. citrophthora.* In these cases not only was the shape and size of the colonies affected but, also, striking morphological changes occurred in the mycelium of the *P.* spp. (Figs. 1, A, B, C, F, and 2). These 2 combinations, therefore, were made the object of further studies.

Experiments were designed to determine, (a) whether the inhibitory substance produced by *Diaporthe citri* was given off in the form of a gas or radiation, (b) effect of the hydrogen-ion concentration in the zone of inhibition, (c) the effects of filtrates containing the metabolic products of *D. citri,* upon the mycelium of the species of Phytophthora, (d) effects of the extracts made from the living mycelium of *D. citri* on the Phytophthora, (e) the behavior of the combinations in liquid cultures using Czapek's and a modification of Coons' (1) liquid medium.

Experiments also were conducted by making inoculations into grape-

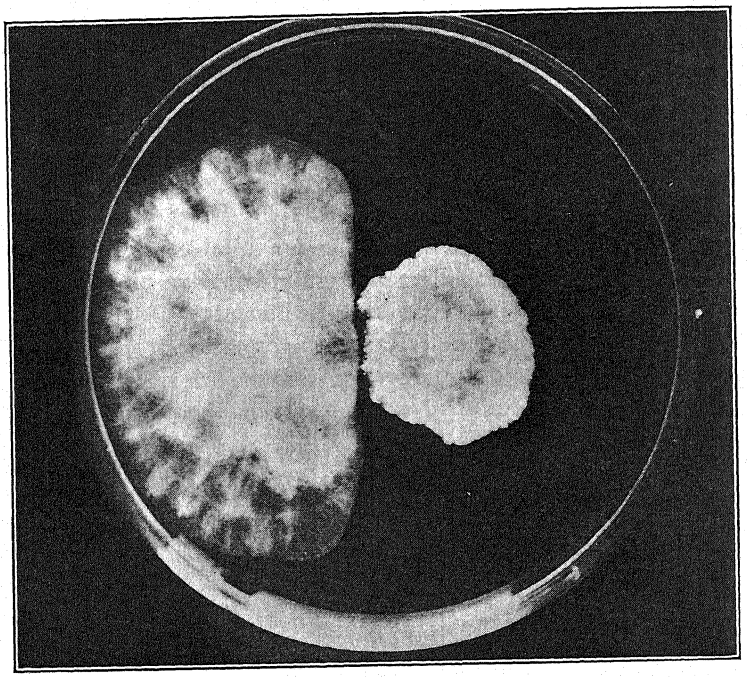


FIG. 2. Colonies of *Diaporthe citri* (right) and *P. citrophthora* (left) after four days of growth on potato-dextrose agar.

fruits, oranges and lemons, to determine whether or not the results already obtained in artificial media could be obtained in fruits.

All the experiments were conducted at room temperatures of 25 to 28° C.

RESULTS

It was found in the preliminary tests, that the various combinations could be grouped into 7 types. Porter (14) described 5 types in similar cases. Four of these were recognized, as well as 3 additional ones not mentioned by him. These 3 types may be described as follows: (a) colonies whose growth stops in front of each other leaving in between a conspicuous straight or somewhat curved free zone, both colonies are checked in growth, (b) a colony that, when approaching that of a different fungus, grows under and then reaches the surface again and continues to grow normally, (one of the colonies is checked) and (c) colonies which when approaching each other are mutually checked in rate of growth, and in one of which striking morphological changes occur in the mycelia. To this last type belong the combinations of *Diaporthe citri* with either of the species of Phytophthora.

The interesting cases of inhibition observed in the combinations of *Diaporthe citri* with either of the species of Phytophthora may be described

as follows: Whenever the edges of the colonies of either *Phytophthora parasitica* or *P. citrophthora* came within 3 mm. of the edge of a colony of *D. citri,* the mycelium of the Phytophthora became strikingly changed, abnormally branched, like a witches' broom and having unusual tendencies to fruiting. (Fig. 1, B and F). On the other hand, colonies of either *P. parasitica* or *P. citrophthora* growing singly in Petri dishes retained their normal shape and growth indefinitely. Also, the colonies of both of the species of Phytophthora, when examined on the edges opposite to the colony of *D. citri,* presented a completely normal appearance.

The fact that inhibition occurred when the colonies were separated by a distance of 3 mm. or more, and the characteristic way in which they stopped in front of each other, made it of interest to determine whether the inhibitory substance was volatile or simply diffusible through the medium (Fig. 2).

For determining the diffusibility of the substance produced by *Diaporthe citri* and its action upon species of Phytophthora, liquid cultures and filtration and extraction experiments were conducted. For determining whether the substance was volatile, a special 2-layer Petri dish was used. The method consisted in the preparation of Petri dishes by pouring potato-dextrose agar in both the dish and its cover. The dishes thus prepared were inoculated with combinations of *D. citri* and *P. parasitica* and *D. citri* and *P. citrophthora.* But one fungus was planted in the dish, while the other was planted in the cover around the center, but so placing the transfers that the 2 resulting colonies finally would be separated by an air space of 3 mm. and a lateral distance of 3 cm. To avoid the possibility of any excretion or secretion of the mycelium of *Diaporthe citri* falling upon the mycelium of either species of Phytophthora, the Petri dishes were maintained constantly in a vertical position.

Observation of all cultures thus prepared showed that both *Phytophthora parasitica* and *P. citrophthora* developed radially to full size and no morphological changes were noticed in their mycelium, proving that if any gas or radiation was given off by *Diaporthe citri* it did not affect the mycelium of Phytophthora.

To determine whether hydrogen-ion concentration was a factor in the inhibition, pH studies were conducted. The pH was determined by the use of the potentiometer. For the determinations, a narrow strip of agar was removed from the zone immediately surrounding the colonies.

The figures obtained in the zones of inhibition seem not to differ enough from those of the margins of normal colonies to warrant any assumption that acidity plays a very important inhibitory rôle.

The results thus far obtained indicated the production of a soluble, non-volatile substance by *Diaporthe citri* as the principal factor in the inhibition of Phytophthora.

TABLE 1.—*The relation of pH to inhibition betweeen Diaporthe citri and Phytophthora species*

Origin of sample for pH determination	Ave. pH
Zone around colony of *Phytophthora parasitica* growing alone	6.21
Zone around colony of *P. citrophora* growing alone	5.74
Zone around colony of *Diaporthe citri* growing alone	5.87
Zone of inhibition between colonies of *D. citri* and *P. parasitica*	5.38–6.20
Zone of inhibition between colonies of *D. citri* and *P. citrophora*	5.24–5.38
Potato dextrose media alone	5.6

The greater possibilities for the diffusion of such a substance in a liquid medium led to the following experiments. The 2 liquid media used were Czapek's solution and an arbitrary modification of Coons' solution (1).

Both Petri dishes and test tubes were used. The Petri dishes, containing 15 cc. of medium, were inoculated by floating bits of mycelium of the combination *Diaporthe citri* and *Phytophthora parasitica* and of *D. citri* and *P. citrophthora* on the liquid; the inocula were placed always at a distance of 3 cm. from each other and in the center of the Petri dishes. The test tubes also were inoculated with both fungi. Controls were run with single inoculations of *P. parasitica* and *P. citrophthora.*

Observations were recorded at intervals of 5 to 7 days for a period of 2 weeks. The following table summarizes the results in Czapek's liquid medium in Petri dishes.

TABLE 2.—*Reaction of Phytophthora parasitica and P. citrophthora with Diaporthe citri in Czapek's and in Coons' solution in Petri dishes*

Fungi used	Culture solution	Observations			
		First week	Second week	Third week	Fourth week
D. citri × *P. parasitica*	Czapek's	x[a]	xxzz	xxzzo	xxzc
P. parasitica (alone)	"	normal	normal	z	zc
D. citri × *P. citrophthora*	"	x	xxzz	xxzzo	xxzc
P. citrophthora (alone)	"	normal	normal	z	zc
D. citri × *P. citrophthora*	Coons'	x	xxzo	xxzzoo	xxzco
P. citrophthora (alone)	"	normal	normal	z	zc

[a] The following legend is used for tables 1 and 2. x—mycelium slightly affected; xx—mycelium strongly affected; o—few oogonia-like bodies; oo—many oogonia-like bodies; c—few chlamydospores; cc—many chlamydospores; z—few zoosporangia; zz—many zoosporangia.

In test tubes, the same results were obtained, but the reproductive structures were less abundantly produced.

The growth of both species of Phytophthora was not abundant; the mycelium spread thinly and less extensively than on the solid medium. In the presence of *Diaporthe citri*, the mycelium of both species of Phytophthora was characteristically affected and the reproductive bodies were always found more abundant in connection with the affected regions.

The growth of the mycelium of *Phytophthora citrophthora* in Coons' solution was very scanty, but the characteristic branching always was observed in the presence of *Diaporthe citri*, and the reproductive bodies were found associated with the affected regions.

It will be noticed in the preceding tables that the colonies of *Phytophthora parasitica* and *P. citrophthora* were not only inhibited and the morphology of the mycelium altered, but, also, that the formation of reproductive bodies, such as zoosporangia, oogonia, and chlamydospores, was stimulated.

THE OOGONIA-LIKE BODIES OF PHYTOPHTHORA CITROPHTHORA

Although these structures were not found in large numbers, all those observed had what appeared to be a distinct oosphere in the center, which distinguished them easily from the chlamydospores. Their size also was smaller; measurements of over 100 giving a range of 17 to 27 μ in diameter. When stained with eosin they became orange yellow in contrast to the reddened mycelium and the reproductive structures. Although the writer is not certain as to the presence of antheridia, in many cases lateral, dark, club-shape structures were seen closely attached to or independent of the oosphere. The chlamydospores in both Czapek's and Coons' media measured from 27 to 44 μ. The zoosporangia, although commonly lemon-shape, varied considerably in shape and size. Their average size in both liquid media was 43×25 μ. The measurements agree with those of Smith (19); Leonian and Geer (10); Guiscafré (6); and Tucker (20). (Fig. 1, E). It is believed that some metabolic product of *Diaporthe citri* is the principal factor in making *P. citrophthora* produce these reproductive structures and especially the bodies here interpreted as oogonia, which have been observed only in cultures where both fungi were present.

In the filtration and extraction experiments, filtrates containing the metabolic products of *Diaporthe citri* were obtained in the following way: test tubes containing Czapek's medium were inoculated with *Diaporthe citri*. Some of the resultant cultures were grown 10 days; others, 20 days. Additional tubes were inoculated with a combination of *D. citri* and *Phytophthora parasitica* and *D. citri* and *P. citrophthora*. These combinations were grown for 20 days. At the end of the alloted growth period, the cultures were filtered through bacteriological filters. The filtrates thus obtained were tested as follows:

(a) Petri dishes were fitted with thin diametrically placed strips of cardboard. One of the halves was filled with potato-dextrose agar, and when this agar was well solidified, the cardboard strips were removed. The other half was then filled with a mixture consisting of ⅔ of one of the filtrates of *Diaporthe citri* and the other third of 1½ per cent plain agar. After this agar was well solidified, *Phytophthora parasitica* and *P. citrophthora* were transferred to Petri dishes thus prepared. The inoculations were made near the center of each dish and 1½ cm. from the dividing line between the 2 kinds of agar.

(b) By filling half of the Petri dishes with potato-dextrose agar in which the species of Phytophthora to be tested was planted and by placing in the other half one of the liquid filtrates.

(c) By making transfers of Phytophthora to tubes containing 5 cc. of the filtrates of *Diaporthe citri.*

Observations made in the 3 different tests after periods of 7 to 10 days showed that the 10-day-old filtrate containing the metabolic products of *Diaporthe citri* had no effect on the mycelia of the species of Phytophthora; that the 20-day-old filtrate produced the characteristic abnormal branching in the species of Phytophthora; and that the inhibitory substance was produced by *Diaporthe citri,* alone, as well as in the presence of the species of Phytophthora.

The filtrates were then subjected to different conditions and tested as described above, on *Phytophthora citrophthora* only.

(a) The filtrate from a 10-day-old culture of *Diaporthe citri* was heated at 50° C. for 18 hours. Result: No effect on the mycelium of *Phytophthora citrophthora.*

(b) The filtrate from a 20-day-old culture of *Diaporthe citri* was heated at 50° C. for 18 hours. Result: Produced the characteristic abnormal branching in the mycelium of *P. citrophthora.* (Fig. 1, G).

(c) The filtrates from 20-day-old cultures of *Diaporthe citri* were autoclaved for 20 minutes at 110° C. and at 20 lbs. pressure, or boiled at 110° C. for 20 minutes in the open. Result: The mycelium of *Phytophthora citrophthora* showed the characteristic abnormal branching when tested with the filtrates thus treated (Fig. 1, D).

These experiments showed that the older the filtrate, the greater the effects produced in the mycelium of *P. citrophthora;* that by heating the 10-day filtrates for 18 hours at 50° C. the inhibitory substance apparently was not sufficiently concentrated to produce any effects; and that the inhibitory substance is thermostable under the conditions indicated.

Extracts of the living mycelium of *Diaporthe citri* were secured by growing the fungus in potato-dextrose agar for 15 days, scraping the mycelium from the agar and grinding it with 15 cc. of sterilized distilled water in a

mortar. The extracts were then run through a bacteriological filter and the filtrate was tested against *Phytophthora citrophthora*. Observations made after 10 days showed almost no effect on the mycelium of Phytophthora. This was to be expected, since, no doubt the inhibitory substance produced by *Diaporthe citri* doubtless had already diffused into the agar, leaving within the mycelium quantities insufficient to have any effect on the mycelium of *P. citrophthora*. However, different results might have been obtained if the extraction had been made at different stages of growth of the fungus.

For the fruit experiments, grapefruits, oranges, and lemons were used. In every case these were selected with attention to uniformity of size, color, and quality. Before inoculation the fruits were first treated with corrosive sublimate (1:1000) and then thoroughly rinsed with sterilized distilled water. Following inoculation the fruits were kept in glass moist chambers at room temperature. The inoculations were performed by making a superficial semicircular wound with a steel needle and placing the inoculum with a platinum needle.

In the first experiment the inoculum of the 2 fungi was placed separately at 3 cm. apart on the periphery of the fruits. Some fruits also were inoculated with each fungus separately to serve as controls.

Observations made after 10 days showed that, although the areas rotted by each fungus could not be well delimited, the mycelium of the Phytophthora was characteristically affected and showed the same abnormal branching observed in Petri dishes and liquid cultures. On the other hand, the fruits inoculated only with Phytophthora showed a completely normal mycelium.

In a second experiment both fungi of each combination were inoculated in the same wound.

Again, the same results were obtained, but this time zoosporangia and a few of the oogonia-like bodies were found in connection with the affected mycelium of *Phytophthora citrophthora* in the fruits inoculated with the combination of *Diaporthe citri* and *P. citrophthora* on the same wound.

In a third experiment this observation was further corroborated by inoculating a large number of oranges. The orange was used because *Diaporthe citri* grew better in it than in either the lemon or grapefruit and because the reproductive structures observed in the preceding experiment were found only in the orange.

In this experiment the reproductive structures were again observed. The controls showed a quite normal mycelium and no reproductive bodies.

A fourth experiment was conducted by inoculating *Phytophthora citrophthora* in fruits in which previously 5 cc. of the filtrate obtained from a 20-day-old culture of *Diaporthe citri* had been injected hypodermically.

Observations after 10 days showed that the mycelium of *Phytophthora citrophthora* was affected when it came in contact with the region in which the filtrate had been injected, but no reproductive structures were observed. Controls similarly treated, except that 5 cc. of sterilized distilled water had been injected into the fruits, showed the mycelium of *P. citrophthora* to be completely normal.

Observations on the fruit were made by crushing bits of tissue from the rotted areas and staining them with eosin. In cases of doubt, isolations were made from the rotted areas to test the identity of the fungus.

In general, the fruit experiments corroborated the results already obtained with the same associations of fungi in Petri dishes.

DISCUSSION AND CONCLUSIONS

Although the writer did not work with particular associations of fungi known in nature, the general behavior of the combinations studied and especially, the striking conditions brought about by a few, have proved definitely that each of these fungi behaves differently when grown in combination with some other fungus.

Opinions differ as to the factor or condition responsible for such inhibitions between organisms. In general, the following explanations have been offered: that it is an enzymatic relationship; an osmotic relationship; an exhaustion of nutrients; a matter of toxicity.

In this work, no attempt was made to determine the chemical composition of the inhibitory substance; but the experiments conducted have demonstrated that the inhibition is not caused by volatile metabolic products of *Diaporthe citri;* that there is no correlation between the hydrogen-ion concentration created by the fungi involved in the zone of inhibition and the inhibition produced; that the substance produced by *Diaporthe citri* is diffusible and filtrable; that the concentration bears a definite relationship to the effects produced on the mycelium of *Phytophthora* spp.; that it is relatively thermostable; and that the substance is produced with constancy, since the same effects were produced in potato-dextrose agar, Czapek's agar, Czapek's and Coons' solution, and on fruits: grapefruits, oranges, and lemons.

The appearance of the mycelium of *Phytophthora citrophthora* was not exactly the same in all the media employed; but this was to be expected since other species of Phytophthora have been found to present variations according to the media in which are grown, (Leonian, 8).

Although zoosporangia and chlamydospores also appeared in liquid cultures and fruits inoculated with *Phytophthora citrophthora,* only, their production and especially that of oogonia is undoubtedly stimulated by the presence of *Diaporthe citri,* since zoosporangia and chlamydospores always

were found in greater numbers and the oogonia were found only in cultures or fruits containing the inocula of both fungi.

The oogonia-like bodies produced by *Phytophthora citrophthora* in association with *Diaporthe citri* are clearly distinguishable from the chlamydospores by size, color of staining reaction, and the presence of an oosphere. The apparent lack of antheridia constitutes no argument against the possible presence of oogonia, here for the first time reported for this species, since the same condition (9) has been found to exist in other species of the genus.

The term inhibition has been used throughout the work, especially in connection with the associations of *Diaporthe citri* and *Phytophthora citrophthora,* since, in all cases, the vegetative growth of the species of Phytophthora is markedly checked. It may seem improper to use such a term when the fungi inhibited formed reproductive structures. The writer, however, believes that the substance produced by *D. citri* actually inhibits the growth of *P. citrophthora* and that the fruiting is merely an attempt on the part of *P. citrophthora* to survive the undesirable conditions introduced by the presence of another fungus, in its environment, at the expense of the checked vegetative growth.

More information is needed, not only as to how the inhibitory substance operates—whether it produces its effects as a result of an osmotic disturbance, or because it is merely toxic to the fungus protoplasm—but, also, as to its chemical nature.

The name *Diaporthe citri* has been used in preference to that of *Phomopsis citri,* since Fawcett (3) found that both *Phomopsis citri* and *Phomopsis californica* had the same perfect stage to which Wolf had already given the name of *Diaporthe citri.*

SUMMARY

Some fungi behave differently when cultured alone than when cultured in association with other fungi.

When certain fungi are cultured in pairs, generally, an inhibition takes place; this may be partial, strong, total, or mutual, checking the rate of growth of one or both fungi and in extreme cases, affecting the morphology and physiology of the fungus inhibited. Cases of acceleration of growth also occur.

Of the 78 combinations tried, those of *Diaporthe citri* with either *Phytophthora parasitica* or *P. citrophthora,* were the most interesting ones, since the growth of the species of Phytophthora was not only checked but also striking morphological and physiological disturbances were produced in the mycelia.

Such factors as metabolic products of *Diaporthe citri* given off in the form of a gas or radiation; habit of growth of the mycelia of the fungi in

the inferior layers of agar; hydrogen-ion concentration produced in the zone of inhibition; and temperature are believed to bear no relation to the inhibition.

A chemical substance contained in the metabolic products of *Diaporthe citri* is believed to be the real cause of the inhibition. This substance was found to be diffusible and filtrable, relatively thermostable, of greater activity in high concentrations, and to be constantly produced by *Diaporthe citri* on a variety of different substrata, just as well in the presence as in the absence of the species of Phytophthora.

Diaporthe citri was found to be the main factor involved in making *Phytophthora citrophthora* form the reproductive structures and, especially, of what are believed to be oogonia.

The chemical substance involved in the inhibition cannot be an enzyme; otherwise it would have been destroyed by a temperature of 110° C.

The growth of the species of Phytophthora is inhibited by a substance produced by *Diaporthe citri*. The formation of reproductive structures in the affected mycelium of *Phytophthora citrophthora* seems to be an indirect response to the stimulus exerted by the presence of *D. citri* in its environment.

BOX 282, MAYAGUEZ, PUERTO RICO.

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ACQUIRED IMMUNITY FROM CUCUMBER MOSAIC IN ZINNIA[1]

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The problem of acquired immunity in plants has long held the attention of phytopathologists, but it is only recently that much consideration has been given the subject in connection with virus diseases. In the last few years, however, considerable evidence that plants may acquire immunity from virus diseases has been obtained. The work of McKinney (7), Thung (13), Salaman (12), Oortwijn Botjes (8), Kunkel (6), Ainsworth (1), and Caldwell (2) has shown that plants infected with one strain of a plant virus become immune from infection with other strains of the same virus. Moreover, evidence presented by Wingard (15) and Price (10) demonstrates that certain species of plants acquire immunity from tobacco ring spot, a disease from which they regularly recover. While considerably more work is necessary to determine the specificity of this acquired immunity, the results assembled up to the present time suggest that the immune reaction is similar to that which obtains in virus diseases of animals, and that it may prove applicable to plant viruses in general. It would be of fundamental importance from both the theoretical and practical standpoints if it could be shown that plants generally acquire immunity from virus diseases and an adequate technique could be found for demonstrating this immunity in a large number of such diseases. Aside from the purely theoretical aspects of the problem, a specific immune reaction would be of considerable value in differentiation and classification of plant viruses.

The reaction of *Zinnia elegans* Jacq. to strains of cucumber mosaic offers a means of testing for a specific cross-immunity reaction with cucumber-mosaic virus. Most strains of the virus cause a mottling disease in zinnia, but one has been found that produces necrotic primary lesions. The problem with which this paper is concerned is that of determining whether zinnia plants infected with mottling strains of cucumber mosaic become specifically immune from infection with the necrotic-type strain of the same virus. The value of using viruses that produce such distinctly different symptoms as mottling and necrosis, for studies on acquired immunity, becomes readily apparent when it is considered that the mottling symptoms of a virus first introduced into a host plant may very well obscure the symptoms of a second mottling virus introduced later, even though the symptoms of the latter be much more severe than those of the former. If, however, the second virus

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is of the necrotic type, it produces lesions that can readily be detected even in leaves showing severe mottling symptoms.

In the experiments to be reported, it was found that zinnia plants mottled by certain strains of cucumber-mosaic virus are immune from infection with the necrotic-type strain of the same virus. On the other hand, the infection of zinnia with certain other viruses, such as tobacco mosaic, does not elicit immunity against this necrotic-type virus. Further evidence of the specificity of the immune reaction in zinnia was obtained by using a strain of tobacco mosaic that produces necrotic primary lesions in this host. Immunity from the necrotic-type tobacco-mosaic virus is conferred by a previous infection with ordinary tobacco- or aucuba-mosaic viruses, but it is not conferred by a previous infection with cucumber mosaic.

MATERIALS AND METHODS

Samples of virus used for inoculation were taken from diseased tobacco (*Nicotiana tabacum* L. var. Turkish) plants. The method of inoculation was the same in all cases. Leaves from diseased plants were macerated in a sterile mortar and a small volume of water was added. The solution thus obtained was taken up on a sterile cheese-cloth pad and was rubbed over the upper surface of several leaves of the plants to be inoculated. The leaves were rinsed with water shortly after inoculation.

The variety of *Zinnia elegans* used in the cross-immunity tests was Lilliput Golden Gem, seed of which were obtained from Peter Henderson and Company, New York. Plants were grown in a greenhouse in which the temperature did not fall below 65° F.

DESCRIPTION OF VIRUSES AND SYMPTOMS PRODUCED IN ZINNIA

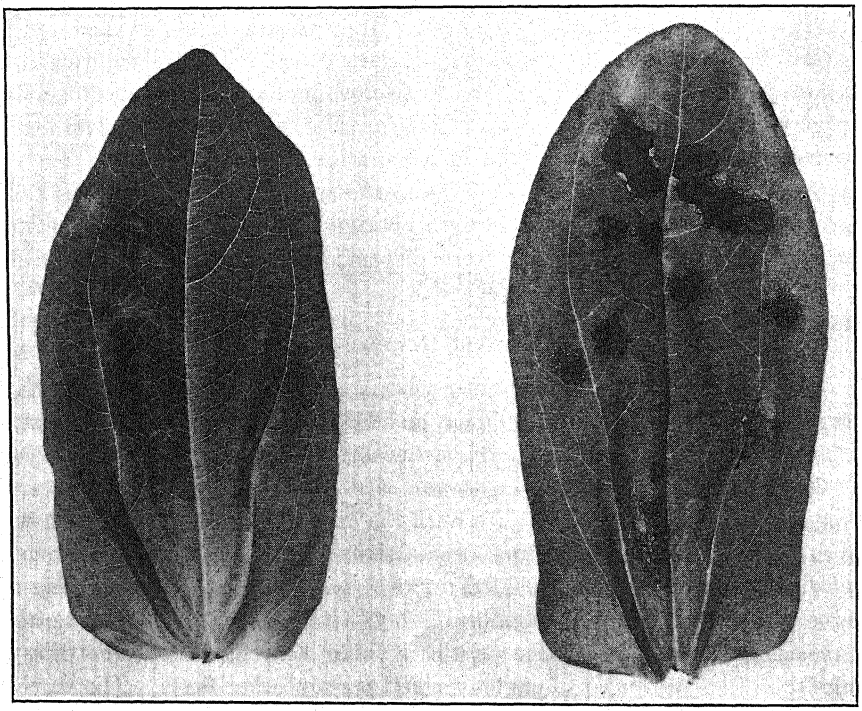
The various strains of cucumber mosaic used in the present study have been described in a previous publication (11). Ordinary cucumber mosaic, referred to here as Porter's cucumber mosaic, was obtained originally from R. H. Porter (9). The other strains are believed to be derivatives of Porter's cucumber mosaic (11). With the exception of strain 6, which will be described later, the cucumber-mosaic viruses tested produce the clearing-of-veins symptom and mottled leaves in *Zinnia elegans*. The clearing-of-veins symptom appears in from 6 to 8 days after inoculation, while mottled leaves make their appearance on the 10th to 12th day. The mottling is similar to that produced in cucumber and certain other hosts. The various strains differ principally in the intensity of the mottling symptoms. Strain 2 produces a brilliant yellow and green mottling, whereas strain 1, strain 9,[2]

[2] This strain was referred to in a previous paper (11) as strain Y. Because of the possibility of confusion with the Y virus of potato, it will hereafter be designated as strain 9.

and Porter's cucumber mosaic produce a mottling of light and dark green. Figure 1 shows the symptoms produced by Porter's cucumber-mosaic virus. Plants infected with any of the mottling strains are considerably stunted but only slightly distorted.

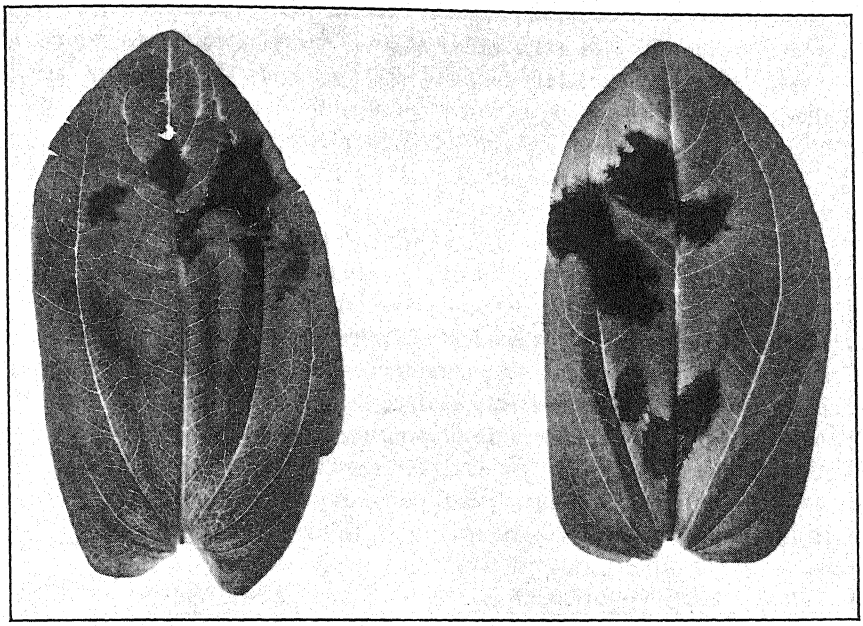
When transferred to zinnia, cucumber mosaic strain 6 induces the formation of bright yellow primary lesions, which appear usually 3 or 4 days after inoculation, soon become necrotic and rapidly increase in size (Figs. 1 and 2). The necrotic lesions are dark brown. In some cases they may involve the entire leaf in which they occur. The necrosis frequently spreads down the petiole into the stem causing elongated, dark brown stem lesions. Occasionally the virus spreads to the tip of the plant, producing necrotic spots similar to those that occur in inoculated leaves. Plants that develop stem lesions usually are killed.

Ordinary tobacco-mosaic virus is readily transmitted to zinnia. Infected plants may develop small, chlorotic, primary lesions in 3 to 5 days after



Photographed by J. A. Carlile

FIG. 1. Lesions produced by inoculation of zinnia leaves with virus of cucumber mosaic strain 6. The leaf on the left was previously mottled by Porter's cucumber mosaic and did not develop lesions. The leaf on the right was a previously healthy control and shows numerous lesions. Porter's cucumber mosaic protects zinnia plants from infection with cucumber mosaic strain 6.



Photographed by J. A. Carlile

FIG. 2. Lesions produced by inoculation of zinnia leaves with virus of cucumber mosaic strain 6. The leaf on the left was previously infected with severe-etch virus. That on the right was a healthy control. Virus of cucumber mosaic strain 6 has produced necrotic primary lesions in both leaves. Severe etch does not protect zinnia plants from infection with cucumber mosaic strain 6.

inoculation, but most plants do not show evidence of primary infection. The clearing-of-veins symptom has been observed in a few plants on the 7th day following inoculation. The most characteristic symptom, however, is a light and dark green mottling of leaves. This is shown rarely by young tip leaves, most conspicuously by leaves just below the tip, and only faintly by older leaves. Plants in which mottling fails to develop show characteristic chlorotic spots similar to those described by Grant (3). Infected plants are usually severely stunted by the disease.

The symptoms of aucuba mosaic in zinnia are in many respects similar to those produced by tobacco mosaic. The mottling pattern is, however, much more conspicuous than is the case with the latter disease. It is composed of areas of various shades of yellow intermingled on a dark green background. Affected leaves frequently develop pale to brilliant yellow zonate ring patterns.

The necrotic-type tobacco-mosaic virus used in the present study was isolated from a yellow-mosaic virus supplied by J. H. Jensen (4) and designated by him as strain 302. In this paper it will be referred to as tobacco

mosaic strain 302A. It is believed to be a derivative of strain 302, which is, in turn, considered to be a derivative of ordinary tobacco-mosaic virus. It produces conspicuous primary lesions in *Zinnia elegans* but does not become systemic. The primary lesions appear in 4 to 6 days after inoculation and enlarge slowly. They are chlorotic at first and become necrotic with age. The necrosis may develop rapidly, the spots being completely necrotic within 6 days, or it may develop more slowly. In many cases the lesions have remained chlorotic for 12 to 14 days after inoculation. Figures 3 and 4 show characteristic lesions produced by strain 302A in zinnia.

The tobacco-ring-spot virus was obtained from S. A. Wingard (15). When transferred to zinnia it produces the clearing-of-veins symptom within 5 or 6 days after infection. This symptom is accompanied by a downward curling or rolling of affected leaves; such leaves tend to blanch and turn yellow at their bases. Leaves developing subsequently may show a mild mottling of green and light green. Ten to 12 days after infection these symptoms are no longer evident; the plants appear healthy or show only a faint mottling. Virus may be recovered readily from the healthy-appearing leaves.

Yellow tobacco-ring-spot virus, obtained from W. D. Valleau (14), produces symptoms in zinnia quite similar to those of Wingard's ring spot. The symptoms are, however, somewhat more severe. Some plants show bright yellow, irregular areas in leaves just below the tip of the plant, while others recover and appear to be healthy after the first onset of the disease.

Severe etch has been described by E. M. Johnson (5). When transferred to zinnia it produces a marked clearing-of-veins symptom (Fig. 2). This symptom persists more or less throughout the course of the disease. In addition, affected leaves are twisted and distorted. They do not develop the typical mottling symptoms characteristic of the mosaic viruses. Infected plants are, however, severely stunted.

THE IMMUNE REACTION IN ZINNIA

Neither cucumber-mosaic strain 6 nor tobacco-mosaic strain 302A produces large numbers of necrotic lesions in leaves of *Zinnia elegans* inoculated by the rubbing method. The maximum number so far produced in a single leaf is approximately 50, while usually only 20 or less are produced. The numbers of lesions vary greatly within a given set of inoculated zinnia plants, and the variation is even greater in different sets of plants. The reason for the variability is obscure, but it is believed that the numbers of lesions may be influenced by the condition of the test plants and by the source of the inoculum. Because of the variation in numbers of lesions in a given set of plants and because of the failure to obtain large numbers of primary infections, it was considered essential to use a large number of control plants

and to repeat the experiments a number of times before drawing definite conclusions. Accordingly, each of the experiments reported here was repeated several times. Except for slight quantitative differences, there was a close agreement between the repetitions of the same experiment.

Leaves Mottled by Cucumber-mosaic Virus are Immune from Infection with Virus of Strain 6. An experiment was performed for the purpose of determining whether or not zinnia leaves that are mottled by any one of 4 different strains of cucumber-mosaic virus are immune from infection with virus of strain 6. Ten young zinnia plants were inoculated with virus of Porter's cucumber mosaic, 10 with virus of strain 1, 10 with virus of strain 2, and 10 with virus of strain 9. Eighteen days after inoculation several leaves on each of the inoculated plants were mottled with green and yellow blotches, while the noninoculated control plants remained healthy. Six of the mottled leaves on each of the diseased plants and 6 comparable leaves on each of the control plants were thoroughly inoculated with virus of cucumber mosaic strain 6. Lesions did not appear in any of the mottled leaves during a period of more than 2 weeks that they were kept under observation. The control plants, with 2 exceptions, developed a number of necrotic primary lesions. The necrotic spots were counted, and the results obtained are given in table 1. The consistency with which lesions developed in healthy leaves, and the complete freedom of mottled leaves from such lesions give conclusive evidence of the immunity of mottled leaves,

TABLE 1.—*Numbers of lesions produced by inoculation of mottled leaves and of healthy leaves of Zinnia elegans with virus of cucumber mosaic strain 6*

Previous condition of test plants	Plant number										Total
	1	2	3	4	5	6	7	8	9	10	
Infected with Porter's cucumber mosaic	0	0	0	0	0	0	0	0	0	0	0
Healthy controls	161	78	105	57	156	38	75	164	108	58	1000
Infected with strain 1	0	0	0	0	0	0	0	0	0	0	0
Healthy controls	13	9	23	10	28	18	8	6	9	12	136
Infected with strain 2	0	0	0	0	0	0	0	0	0	0	0
Healthy controls	10	16	6	21	0	11	8	4	18	3	97
Infected with strain 9	0	0	0	0	0	0	0	0	0	0	0
Healthy controls	9	11	3	10	12	6	0	16	7	8	82

in spite of the fact that only a small number of lesions was produced in some of the controls. The immunity against strain 6 afforded by a previous infection with Porter's cucumber mosaic is illustrated in figure 1. It is concluded that zinnia plants that are mottled by Porter's cucumber mosaic, cucumber mosaic strain 1, strain 2, or strain 9 are immune from infection with virus of cucumber mosaic strain 6.

Invasion of Protecting Viruses Necessary for Complete Immunization of Plants against Strain 6. In the experiment just reported, virus of the mottling strains of cucumber mosaic was allowed to become thoroughly systemic before testing for immunity. It appeared desirable to determine whether or not plants that had been infected for a shorter period of time and in which the virus had not become thoroughly systemic would likewise be immune from infection with strain 6. Ten young zinnia plants were inoculated with Porter's cucumber-mosaic virus, 10 were inoculated with strain 1, 10 with strain 2, and 10 with strain 9. An equal number of plants were kept uninoculated and served as controls. On the 11th day after inoculation the plants showed the clearing-of-veins symptom. Four leaves near the tip of each of the diseased plants were then thoroughly inoculated with virus of strain 6. An equal number of control leaves were inoculated with the same virus samples. Lesions appeared in the test plants in leaves that showed the clearing-of-veins symptom, and also in healthy appearing leaves. Table 2 shows the total numbers of lesions produced in the test and

TABLE 2.—*Numbers of lesions produced by inoculation of zinnia plants with virus of cucumber mosaic strain 6. The test plants had been previously infected with ordinary cucumber mosaic and were just showing the clearing-of-veins symptom. The controls were previously healthy plants*

Virus tested	Number of lesions	
	Test plants	Controls
Porter's cucumber mosaic	24	186
Strain 1	12	170
Strain 2	21	105
Strain 9	10	42

control plants. An examination of the table reveals that lesions were far more numerous in the previously healthy plants than in previously infected plants. Some immunity was shown by the test plants, but it was not complete. The results indicate that acquired immunity from strain 6 is confined to tissues actually invaded by the protecting virus.

As a further check to the experiment, the inoculated leaves showing lesions were removed and the diseased plants allowed to become thoroughly

mottled. On the 28th day after the first inoculation, both diseased and control plants were inoculated with virus of strain 6. None of the plants infected with either Porter's cucumber mosaic, strain 1, strain 2, or strain 9 developed lesions. A total of 136 lesions appeared in the controls.

Failure to Immunize Inoculated Leaves. It has been shown by Kunkel (6) that leaves of *Nicotiana sylvestris* Speg. and Comes inoculated with tobacco-mosaic virus become immune from aucuba-mosaic virus. An experiment was, therefore, undertaken in order to determine whether zinnia leaves inoculated with Porter's cucumber-mosaic virus likewise become immune from cucumber mosaic strain 6. Six leaves on each of 30 healthy zinnia plants were thoroughly rubbed with juice containing Porter's cucumber-mosaic virus. At the same time, 6 leaves on each of 30 similar plants were rubbed with water. The latter set of 30 plants was used as controls. After 6 days the rubbed leaves of each of the 60 plants were inoculated with cucumber mosaic strain 6. A total of 1118 necrotic lesions developed in the leaves that were previously inoculated with Porter's cucumber mosaic. A total of 1345 necrotic lesions appeared in the controls. The results show that zinnia leaves previously inoculated with ordinary cucumber-mosaic virus were not fully protected from infection with strain 6. The results of a previous experiment have shown that the invasion of tissues by the protecting viruses is necessary for their complete immunization against strain 6. It is, therefore, believed that the failure to obtain immunization of leaves inoculated with Porter's cucumber mosaic resulted from inability to produce the numerous primary infections that are necessary for complete invasion of the tissues of inoculated leaves.

SPECIFICITY OF THE IMMUNE REACTION

The failure of zinnia plants mottled by certain strains of cucumber-mosaic virus to develop lesions when inoculated with virus of cucumber mosaic strain 6 demonstrates that such plants are immune from the latter virus. It seemed desirable, however, to determine whether this immunity is or is not specific. Two types of experiment were, therefore, conducted. Firstly, tests were made with a number of viruses believed to be unrelated to cucumber mosaic in order to determine whether or not they protect zinnia plants from infection with cucumber mosaic strain 6. Secondly, reciprocal tests were made to determine whether or not zinnia plants mottled by cucumber mosaic, or certain other viruses, are completely protected against infection with the necrotic-type tobacco mosaic, strain 302A.

Viruses Believed to be Unrelated to Cucumber Mosaic do not Confer Immunity from Strain 6. Sets of 10 young zinnia plants were inoculated with the following viruses: tobacco mosaic, aucuba mosaic, severe etch, tobacco ring spot, and yellow ring spot. An equal number of similar plants

were not inoculated and served as controls. All the inoculated plants became diseased and developed symptoms characteristic for the virus with which they were infected. Eighteen days after inoculation, 6 infected leaves on each of the diseased plants and 6 leaves on each of the healthy controls were rubbed with juice containing virus of cucumber mosaic strain 6. Necrotic lesions developed in the diseased plants, as well as in the controls. The numbers of lesions are shown in table 3. From this table it will be seen

TABLE 3.—*Numbers of lesions produced by inoculation of infected leaves and of healthy leaves with virus of cucumber mosaic strain 6*

Previous condition of test plants	Plant number										Total
	1	2	3	4	5	6	7	8	9	10	
Infected with tobacco mosaic	7	0	0	9	1	2	1	1	0	4	25
Healthy controls ...	1	11	18	7	6	1	17	4	9	24	98
Infected with aucuba mosaic	12	22	9	12	17	38	12	1	5	7	135
Healthy controls ...	22	4	6	1	20	1	4	19	10	14	101
Infected with severe etch	6	0	3	1	5	6	1	9	0	4	35
Healthy controls ...	1	4	4	1	0	3	5	7	6	1	32
Infected with tobacco ring spot..	3	19	5	3	13	2	5	15	9	1	75
Healthy controls ...	10	17	24	6	13	12	41	8	12	18	161
Infected with yellow ring spot.....	10	13	13	4	20	8	15	10	8	9	110
Healthy controls ...	44	27	10	16	68	9	21	4	12	17	228

that the plants having tobacco mosaic, tobacco ring spot, and yellow ring spot developed fewer lesions than the controls, but that those infected with aucuba mosaic showed more lesions than the corresponding controls. In the case of most of the viruses tested, the lesions appeared equally early in both the diseased and healthy plants. However, in the case of severe etch, the lesions in the diseased plants required 8 days to become completely necrotic as compared with 5 days for the lesions in the controls. Infection of zinnia plants with tobacco mosaic, aucuba mosaic, tobacco ring spot, yellow ring spot, or severe etch did not confer immunity against cucumber mosaic strain 6. The failure of severe-etch virus to immunize zinnia plants against cucumber mosaic strain 6 is illustrated in figure 2. The results indicate that

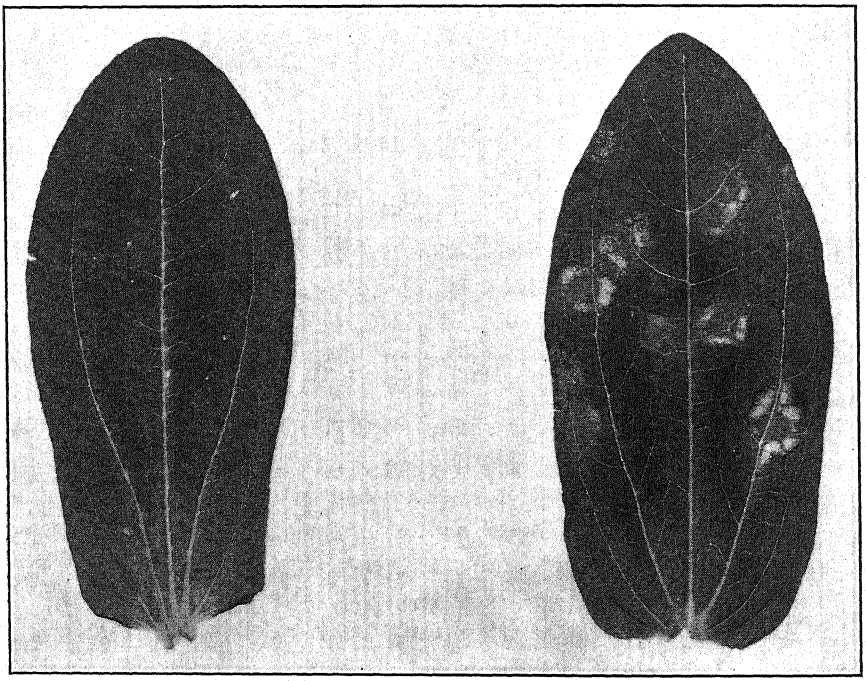
the immune reaction against virus of strain 6 is confined to plants infected with strains of cucumber-mosaic virus.

Reciprocal Cross-immunity Tests in Zinnia. In conducting the reciprocal cross-immunity tests, use was made of tobacco mosaic strain 302A, a necrotic-type virus in zinnia. Young zinnia plants were divided into sets of 10 each and inoculated with the following viruses: tobacco mosaic, aucuba

TABLE 4.—*Numbers of lesions produced by inoculation of infected leaves and of healthy leaves with virus of tobacco mosaic strain 302A*

Previous condition of test plants	Plant number										Total
	1	2	3	4	5	6	7	8	9	10	
Infected with tobacco mosaic	0	0	0	0	0	0	0	0	0	0	0
Healthy controls	15	10	18	8	18	3	29	26	15	9	151
Infected with aucuba mosaic	0	0	0	0	0	0	0	0	0	0	0
Healthy controls	16	1	3	21	10	32	44	3	14	–	144
Infected with cucumber mosaic	0	13	1	1	8	4	5	16	4	2	54
Healthy controls	0	4	2	10	10	6	4	7	9	8	60
Infected with strain 1	11	10	0	19	24	11	3	9	30	25	142
Healthy controls	13	11	36	45	19	13	13	50	19	15	234
Infected with strain 2	2	4	12	9	39	13	5	0	8	23	115
Healthy controls	18	45	3	8	21	8	13	32	8	13	169
Infected with strain 9	20	2	7	15	5	11	5	11	10	0	86
Healthy controls	17	16	14	18	21	13	33	3	32	6	173
Infected with tobacco ring spot	14	41	29	17	0	18	9	16	16	13	173
Healthy controls	51	38	15	45	31	3	13	13	3	40	252
Infected with yellow ring spot	21	38	12	34	34	11	63	34	6	20	273
Healthy controls	27	30	13	21	28	10	35	34	9	22	229
Infected with severe etch	13	10	10	7	28	4	6	15	30	11	134
Healthy controls	20	11	33	7	10	6	22	14	13	38	174

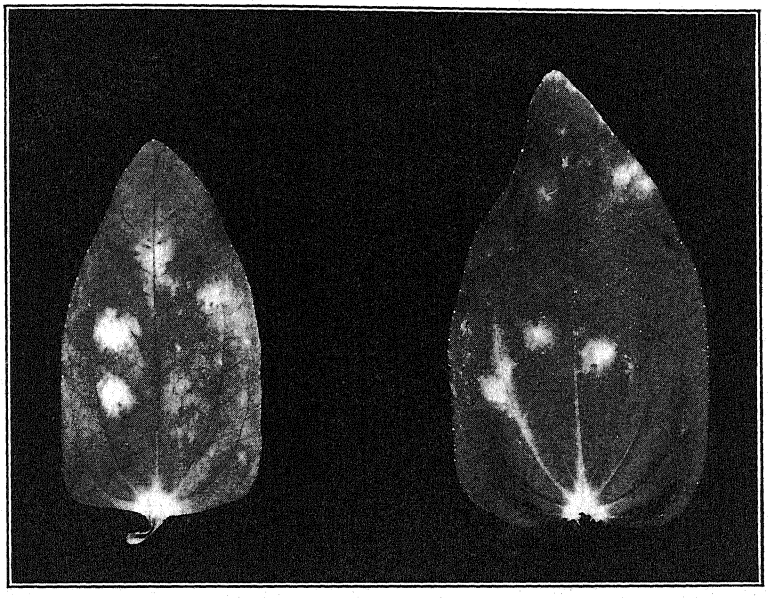
mosaic, Porter's cucumber mosaic, cucumber mosaic strain 1, cucumber mosaic strain 2, cucumber mosaic strain 9, tobacco ring spot, yellow ring spot, and severe etch. All the inoculated plants became infected and developed the usual symptoms. An equal number of similar plants left non-inoculated and used for controls remained healthy. Sixteen days after inoculation, 4 infected leaves on each of the diseased plants and 4 leaves on each of the controls were inoculated with virus of tobacco mosaic strain 302A. No lesions developed in any of the plants infected with either tobacco mosaic or aucuba mosaic, but the lesions were numerous in the controls and in the plants infected with the other viruses, as shown in table 4. The results show, firstly, that zinnia plants that are infected with tobacco- or aucuba-mosaic virus become immune from infection with virus of tobacco mosaic strain 302A, and, secondly, that zinnia plants infected with ordinary cucumber-mosaic virus or with any one of 3 other strains of this virus, or with tobacco-ring-spot, yellow-ring-spot, or severe-etch virus do not acquire immunity from virus of tobacco mosaic strain 302A. Figure 3 illustrates



Photographed by J. A. Carlile

FIG. 3. Lesions produced by inoculation of zinnia leaves with virus of tobacco mosaic strain 302A. The leaf on the left was previously infected with aucuba mosaic and did not develop lesions. The leaf on the right was previously healthy and developed a number of lesions. Aucuba mosaic protects zinnia plants from infection with tobacco mosaic strain 302A.

the immunity against virus of tobacco mosaic strain 302A induced by a previous infection with aucuba-mosaic virus. The failure of cucumber-mosaic virus to elicit immunity against virus of tobacco mosaic strain 302A is illustrated in figure 4. The results bring additional evidence that the immune reaction in zinnia is specific.



Photographed by J. A. Carlile

FIG. 4. Lesions produced by inoculation of zinnia leaves with virus of tobacco mosaic strain 302A. The leaf on the left was previously infected with Porter's cucumber mosaic. The leaf on the right was previously healthy. Both leaves have developed several lesions. Porter's cucumber mosaic does not protect zinnia plants from infection with tobacco mosaic strain 302A.

DISCUSSION

The results presented in this paper are based, for the most part, on infections resulting in small numbers of lesions. They are, nevertheless, considered to be significant. The tests show that zinnia plants infected with mottling strains of cucumber-mosaic virus become immune from infection with a necrotic-type strain of this virus. They show, moreover, that this immunity is specific and does not extend to any of the unrelated groups of plant viruses. The immune reaction in zinnia allows a classification of the 5 cucumber-mosaic viruses into a distinct group and the 3 tobacco-mosaic viruses into a second distinct group. Further evidence is needed, however, for determining the relationships of severe etch, tobacco ring spot, and

yellow tobacco ring spot. None of these viruses conferred immunity against either tobacco mosaic or cucumber mosaic.

The mechanism by which zinnia plants become immune from cucumber mosaic strain 6 has not been revealed. It is known, however, that this immunity is associated with the presence of the protecting virus in tissue areas that acquire immunity. Uninvaded leaves on plants infected with mottling-type strains of cucumber-mosaic virus are susceptible to infection with the virus of strain 6. For this reason, it is necessary to determine whether or not the virus under test has become thoroughly systemic before definite conclusions can be reached. In the present work in which zinnia plants were inoculated with different viruses, the infected plants were allowed to become thoroughly invaded before being tested for immunity against either cucumber mosaic strain 6 or tobacco mosaic strain 302A.

Zinnia elegans is not an ideal plant for use in a study of acquired immunity from cucumber- or tobacco-mosaic viruses. It does, however, offer certain advantages that are not possessed by any other host plant, so far as known. The greatest advantage lies in the fact that within both the cucumber- and tobacco-mosaic groups strains have been found that produce primary necrotic lesions in zinnia, whereas the other strains tested produce mottling diseases. This difference in reaction is admirably adapted to cross-immunity studies and allows a severe test to be made of the specificity of the reaction. Zinnia also possesses the advantage of being susceptible to a number of different virus diseases and thereby allows a further extension of the work on differentiation and classification. On the other hand, the susceptibility of zinnia to the viruses employed is much lower than might be desired. This difficulty has been overcome by using a large number of plants and by repeating the tests a number of times.

SUMMARY

A description is given of the symptoms produced in *Zinnia elegans* by a necrotic-type cucumber-mosaic virus and by a necrotic-type tobacco-mosaic virus.

Zinnia plants that are mottled by any one of 4 different strains of cucumber-mosaic virus become immune from infection with a necrotic-type strain of this virus, but are susceptible to a necrotic-type tobacco-mosaic virus. Similarly, plants that are mottled by tobacco- or aucuba-mosaic virus become immune from a necrotic-type tobacco-mosaic virus, but do not develop immunity against a necrotic-type cucumber-mosaic virus. On the other hand, plants infected with tobacco-ring-spot, yellow-ring-spot, or severe-etch virus do not acquire immunity either from the necrotic-type cucumber-mosaic virus or from the necrotic-type tobacco-mosaic virus.

Evidence is thus obtained of the specificity of the immune reaction in zinnia for both cucumber- and tobacco-mosaic viruses.

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MORPHOLOGIC STUDIES OF SEPTORIA LYCOPERSICI[1]

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(Accepted for publication December 1, 1934)

This study was not occasioned by belief that the existent morphologic investigations of *Septoria lycopersici* Speg., the tomato leaf-spot fungus, were inadequate. It was, instead, purported to confirm, if possible, certain developmental features of the fungus and perhaps to increase the present knowledge of the organism by additional facts that might present themselves during the course of investigation.

These morphologic observations include: (1) character of the vegetative mycelium; (2) developmental stages of pycnidial and pycnospore formation; (3) comparative studies of pycnospore germination in hanging-drop culture and on the host plant; and (4) relationship between the fungus and the host tissue, including infection penetration.

MATERIALS AND METHODS

The fungus used in this study was isolated from garden-grown tomato plants in Urbana, Illinois. The plants were moderately infected with Septoria leaf spot, and on the date of collection, October 5, 1931, the leaves bore numerous spots containing mature pycnidia exuding masses of pycnospores.

A single spore isolation of the fungus was made according to the method outlined by Davis (2), and the resultant cultural growth was that used for inoculation purposes.

Separate leaflets of several potted tomato plants were inoculated with a single loopful of a rather concentrated pycnospore suspension in sterile distilled water. The method employed by Levin (6) of placing a small, thin piece of cotton over the point of inoculation also was adopted. The cotton not only served as an exact means for locating the inoculation point, but also prevented the removal of pycnospores due to the condensation of moisture on the leaves, since the inoculated plants were placed in humidity cases for infection development.

Histological material was secured by taking 1 sq. cm. of leaf tissue from the inoculated points for 12 successive days. The tissue was killed

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[2] The writer wishes to express grateful acknowledgment for the kindly suggestions and indispensable advice given by the late Professor F. L. Stevens under whose supervision this work was done.

and fixed in strong chromo-acetic-acid solution, and the chloroform method of infiltration was followed. Paraffin sections of the imbedded leaf tissue were cut 5 to 8 μ in thickness.

To secure differential staining of fungus and host tissue, Pianeze III B (8), and Dickson's stain (3) were tried. Effective differentiation could not be secured with the former stain, inasmuch as the parenchymatous

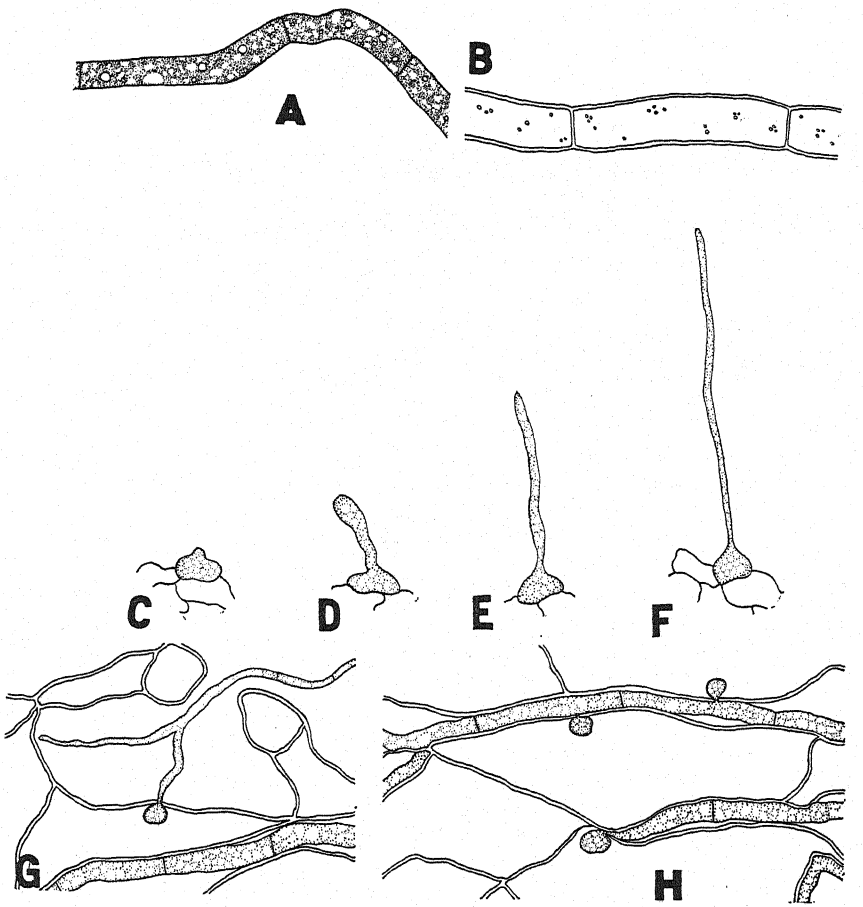


FIG. 1. A–B. Two types of mycelium occurring in the host tissue. ×1250. A. Hyaline, thin-wall type. B. Brown, thick-wall type. C–F. Progressive stages of pycnospore formation. ×1250. C. Initial stage showing the small, papillate protrusion from a cell of the undifferentiated sporogenous tissue. D. and E. Successive stages of the elongative basipetal development of pycnospores. F. Nearly mature pycnospore prior to its constrictive separation from the sporogenous cell. G. Cross section of stomatal penetration by germinating pycnospore on the upper leaf surface after 60 hours. ×1000. H. Intercellular mycelium of the fungus showing penetration of the host cells by blunt, globular haustoria. ×1000.

tissue of the tomato leaf did not retain the malachite green, but stained red, like the fungus. Excellent differentiation was secured, however, with Dickson's combination stain.

MYCELIUM

The mycelium of *Septoria lycopersici* is of 2 types: (1) the hyaline, thin-wall type (Fig. 1, A), and (2) the brown, thick-wall type (Fig. 1, B).

The hyaline mycelium is characteristic of the young and actively growing hyphae, and predominates during the early progress of the fungus through the leaf tissue succeeding initial infection. The sporogenous tissue within the pycnidium also consists of this type of mycelium. The hyphal walls are quite thin, hyaline, and infrequently septate, though occasionally close septation occurs. Protoplasmic contents are densely granular, vacuolated, and contain numerous small oil globules. The diameter of the hyaline mycelium ranges from 1.2 to 5.8 μ.

The brown mycelium is characteristic of the older mycelium, and is also the type that enters into the formation of the pycnidial wall. This mycelium is infrequently branched, and usually exhibits close septation. Its brown hyphal walls are about twice as thick as those of the hyaline mycelium. Protoplasmic contents are almost imperceptible, though occasionally large vacuoles and a few scattered oil globules occur. A diameter of 2.2 to 5.8 μ has been noted for this type of mycelium.

PYCNIDIAL FORMATION

Levin studied the mode of pycnidial formation of *Septoria lycopersici,* and found it to be symphyogenous A study of the fungus in the present investigation confirms the report of Levin, and in no instance has evidence of meristogenous development been observed.

In the initial stage of pycnidial formation, several hyphae aggregate and intersect in the leaf tissue (Fig. 2, A). Here the hyphal tips are just beginning to bend and intertwine preparatory to the formation of a pycnidial primordium (Fig. 2, B). In this early primordial stage, the intertwining hyphae have formed a conglobate mass of hyphal tissue that already has begun to assume the appearance of pseudoparenchyma. Figure 2, C, shows a somewhat later primordial stage. Continued division and aggregation of hyphal cells have occurred, and, in this instance, in close proximity to the vascular tissue of the leaf. The next stage to be observed in pycnidial formation is the development of a pycnidial chamber (Fig. 2, D). This central cavity is just beginning to form, and all observational evidence indicates that it results from a schizogenous process in which the central hyphae or hyphal cells are pulled apart because the peripheral primordial growth is greater than the interior growth. Figure 2, E, shows

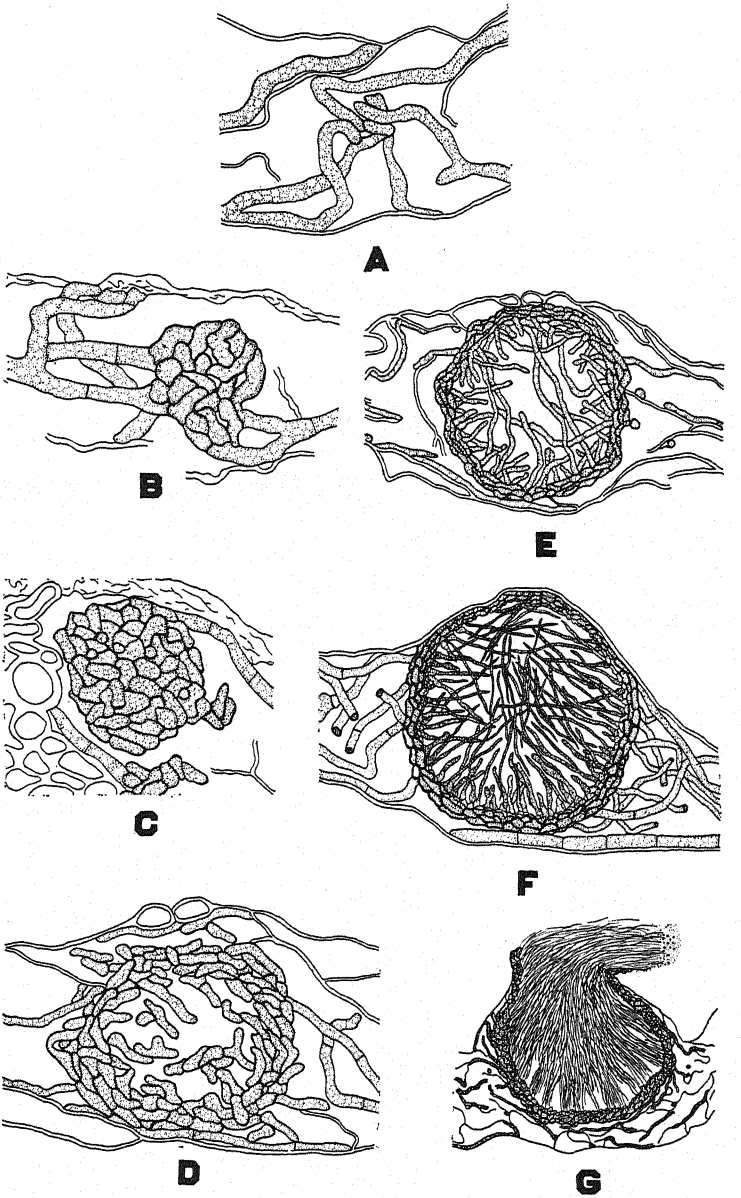


FIG. 2. Developmental stages in the formation of pycnidia. A. Aggregation and intersection of hyphae preparatory to the symphyogenous formation of the pycnidial primordium. ×1000. B. Early stage in the development of the pycnidial primordium. ×1000. C. Later stage in the formation of a pycnidial primordium showing the continued development of pseudoparenchymatous tissue. ×550. D. Young pycnidium showing the appearance of the pycnidial chamber during its early formation. ×900. E. Later stage of pycnidial development showing the pycnidial chamber prior to ostiolar formation, and with several hyphae still extending across it. ×500. F. Pycnidium in the initial stage of pycnospore formation previous to ostiolar formation. ×400. G. Fully mature pycnidium, in which extrusion of pycnospores has occurred through the wide ostiole. ×225.

this central pycnidial cavity well-developed, though mycelial strands still extend across the chamber. In this stage a definite pycnidial wall is seen to exist and to consist of compacted peridial cells. Figure 2, F, shows a pycnidium in which pycnospore formation has just begun, and numerous, separate pycnospores already occur in the pycnidial chamber. This pycnidium also shows a stage of development that will be treated further in the subsequent discussion of ostiolar formation. Figure 2, G, represents the most advanced stage of pycnidial development, and this fully mature pycnidium is characterized by the exudation of great masses of pycnospores through the conspicuously wide ostiole.

Archer (1) studied the morphological characters of several Sphaeropsidales in culture, including *Septoria lycopersici,* and ascertained some additional facts relative to pycnidial formation that were unreported by Levin. He found that, just preceding pycnospore formation, the hyphae extending across and through the pycnidial chamber undergo dissolution to form a dense, vacuolated, gelatinous substance. This gelatinous material, which fills the pycnidial cavity, is displaced when pycnospores are formed and forced into it.

In the present investigation a pycnidial stage in which a general gelatinization of hyphae occurred was not observed. Close examination of several pycnidial stages, prior to pycnospore formation, did show, however, fragmental hyphae evidencing dissolution. These hyphal fragments were quite swollen and irregular, and portions of them were almost imperceptible, since they were in a partial state of disintegration. Another fact tending to substantiate hyphal gelatinization was the release of a hyaline, granular substance at the same time pycnospores were exuded through the ostiole. This substance (Fig. 2, G) was forced out together with the pycnospore mass, and apparently represents the disintegrated hyphal material.

Diedicke (4), supported by the investigations of Klebahn (5) and Potebnia (7), reported that some species of Septoria have more or less broad, open-top fructifications from which a peridium arises and partially surrounds the inner sporogenous tissue. Potebnia has designated such a structure, a pseudopycnidium, and Diedicke excluded all forms lacking this pseudopycnidium as true Septorias. According to Diedicke, *Septoria lycopersici* shows neither a pseudopycnidial stage nor a preformed ostiole at any time during its development, and, therefore, could not be classified as a Septoria. Archer is opposed to this pseudopycnidial distinction by Potebnia and Diedicke, and states: "The writer considers there has not been a single fact presented to prove that the pycnidial wall does form in this way or that the fruiting body of Septoria is any more 'pseudopycnidial' than those of Sphaeropsis of Phoma species."

Pycnidial formation of *Septoria lycopersici* is not definitely positioned in the host tissue, as is reported for some species of Septoria. Weber (9) found that *Septoria nodorum* Berk., and *Septoria tritici* Desm. developed pycnidia only in the substomatal chambers. In a few instances this has been observed also to occur with *Septoria lycopersici.* However, the pycnidia of this fungus also form in the spongy tissue of the leaf and, frequently, directly beneath stomata (Fig. 2, D and E).

The pycnidia do not form simultaneously, but various stages of development occur successively. Thus, pycnidia were first observed forming 6 days after inoculation, but they were also still continuing to form in material examined 12 days after inoculation. On this latter date, mature pycnidia exuding spores were also present in the same material.

OSTIOLAR FORMATION

Levin (6) stated that two forces operated in forming the ostiole of *Septoria lycopersici:* (1) that brought about by the tension between the epidermis and enlargement of the pycnidium, and (2) that exerted by the pycnospore mass on the pycnidial wall at the point of greatest tension.

Archer's examinations (1) of the ostiolar formation of the fungus led him to believe that another factor also is involved in the process, namely, a dissolving factor. His evidence consists of the presence of separated, brown, thick-wall primordial cells lying free within or else lining the pycnidial cavity. These cells exhibited every appearance of having been partially dissolved. This dissolving action, combined with the internal force exerted on the pycnidial wall, would account for the wide and irregular ostiole characteristic of *Septoria lycopersici.*

The conclusions of Levin (6) concerning ostiolar formation have apparently been confirmed in the present study, but no evidence has been observed to substantiate the lysigenous action reported by Archer. It is not to be implied, however, that such lysigenous action does not occur, but rather that it was not observed in the material from which this present study was made.

It is obvious that the pycnidium shown in figure 2, F, is exerting a tension on the epidermal layer of the leaf, since this is greatly extended by enlargement of the pycnidial wall. This force, together with that to be exerted by the great mass of pycnospores beneath the point of ostiolar formation, would, however, seem sufficient to rupture the pycnidial wall, even if no lysigenous action were involved. That ostiolar formation involves a tensional relationship, was substantiated by cultural growth of the fungus on agar by both Levin (6) and Archer (1). Often, the weakest point of the pycnidial wall was submerged in the agar, and pycnospore

masses were frequently observed to extrude into the agar substratum instead of above its surface.

PYCNOSPORE FORMATION

The pycnospores of *Septoria lycopersici* are filiform, curved or straight, and their walls are thin. They are pluriseptate, consisting of 3 to 9 cells, and their ends are pointed to globose. Protoplasmic contents are vacuolated, but the cytoplasm itself appears smooth and homogeneous. The pycnospores range in size from 60–120 × 2–4 μ.

Progressive stages in the formation of pycnospores are shown in figure 1, C–F. Cells forming the undifferentiated sporogenous tissue at the periphery of the pycnidial cavity first push forth a small, short, papillate protrusion (Fig. 1, C). This protrusion continues to elongate (Fig. 1, D and E), and, ultimately, as the pycnospore approaches maturity, separation from the sporogenous cell occurs by basal constriction (Fig. 1, F). After a pycnospore is constricted, other pycnospores may form from the same sporogenous cell. Thus, a continuous basipetal formation of pycnospores occurs, filling the entire pycnidial chamber.

PYCNOSPORE GERMINATION

Levin studied the pycnospore germination of *Septoria lycopersici* in hanging-drop cultures of sterile water and on various media. No comparative studies were made of germination on the host plant.

With this purpose in view, germination studies were made of pycnospores in sterile water in Van Tieghem cells and also in sterile water on both the upper and lower leaf surfaces. The hanging-drop cultures were placed in humidity cases with the plants, so that temperature conditions were the same in all instances. The pycnospores were examined at the end of 24, 36, and 48 hours.

A few pycnospores exhibited initial stages of germination at the end of 36 hours. Germination was quite general at 48 hours, and this time best represents that required for germination. Even then, however, pycnospores were observed that showed no signs of germination, while others were just exhibiting the formation of germ tubes.

The first noticeable characteristic to be observed during germination is a swelling of the pycnospore due to imbibition of water. Swelling is greater for diameter than for length of the pycnospore, and a germinating pycnospore usually has a diameter 2 to 4 times greater than that of a nongerminating one. A vacuolated condition of the protoplasm is also a prominent feature during early germination.

Germ-tube formation occurs on both the inner and apical cells of the pycnospore (Fig. 3). Apical cell germination, however, is more nearly

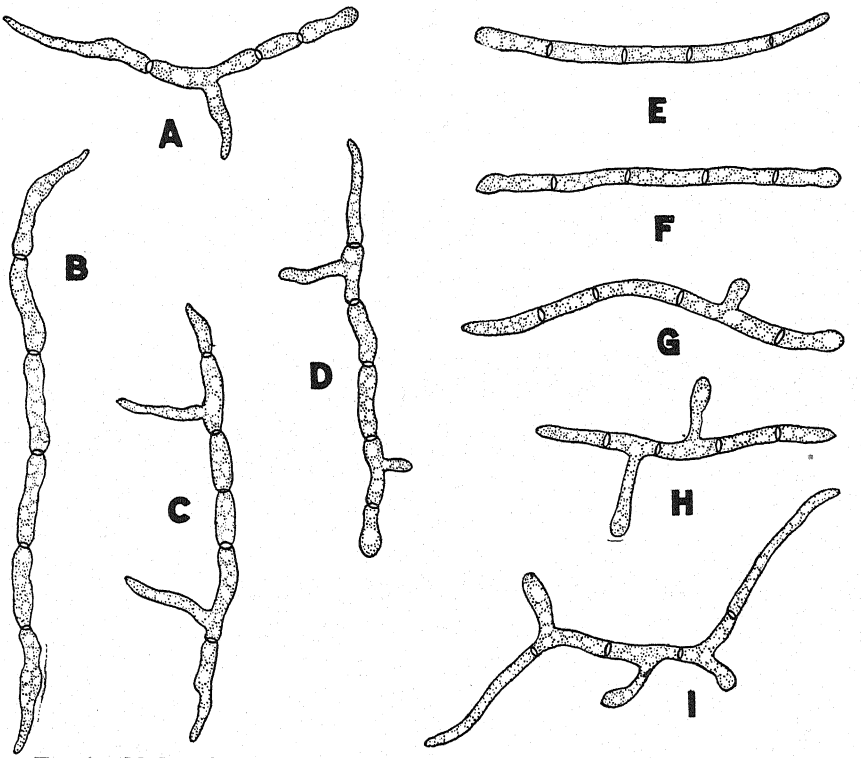


FIG. 3. Mode of pycnospore germination after 48 hours. ×1250. A–D. Germination in a Van Tieghem cell. E–I. Germination on the under side of a tomato leaf. Germ tubes of these pycnospores are rather blunt as compared with those produced by pycnospores germinated in hanging-drop culture, and the septa are not so prominently constricted.

comparable to a simple hyphal extension or elongation of the cell, since the apical cells were never observed to form lateral germ tubes. Elongation of apical cells usually is preceded by a globose enlargement at the end of the cell, and lateral germ-tube formation is characterized by a lateral wall protuberance that ultimately extends forth as a short germ tube.

Various combinations in the formation of lateral germ tubes may occur during germination. Either one cell or several cells of the pycnospore may form a germ tube, and these germ tubes may occur on the same side or on both sides of the pycnospore.

Anastomosis between 2 germinating pycnospores was frequently observed, and the anastomosing tube formed between any of the inner cells of the 2 pycnospores.

The mode of pycnospore germination, as observed during this study, is essentially the same as that reported by Levin (6). Some differences were to be noted, however, between the germination of pycnospores in

hanging-drop culture and those germinated on the host plant. As may be seen in figure 3, A to D, which represents pycnospore germination in hanging-drop culture, the germ tubes usually are characterized by rather pointed or tapering ends. The individual cells of these pycnospores are conspicuous and impart a decidedly jointed appearance to the pycnospores. The germ tubes of pycnospores germinated on the host plant (Fig. 3, E to I) are quite rounded and rather blunt. Constriction at the septa is lacking in these pycnospores.

No difference was to be noted between the germination of pycnospores on the upper and lower leaf surfaces.

INFECTION PENETRATION

The mode of infection penetration of *Septoria lycopersici* has been determined as stomatal (Fig. 1, G), as Levin (6) likewise reported. In no instance was direct infection penetration observed to occur through the cuticle and epidermal cells.

It has not been possible to follow the progressive development of the mycelium through the leaf, but the chief facts of the relationship between the fungus and the host tissue have been determined.

The mycelium is intercellular (Fig. 1, G and H), and penetrates the host cells by means of blunt, globular haustoria (Fig. 1, H). Haustoria have been observed only on the hyaline type of mycelium, and their protoplasmic contents are densely granular. The penetration hypha, after entering the substomatal chamber (Fig. 1, G), may branch and effect immediate haustorial penetration of adjacent cells. Again, the mycelium may begin intercellular extension through the leaf after infection penetration. The mycelium occurs in all parts of the leaf, but it is most abundant in the mesophyll region. Also, it is not wholly confined to the visible leaf-spot area, but extends short distances into the adjacent tissue.

Vascular tissue restricts mycelial progress to a certain extent, and hyphae sometimes terminate in this region, as shown by the hypha (Fig. 2, C) just beneath the pycnidial primordium. Hyphae, however, may occur also in the tracheae. One vessel was observed that was almost completely filled by the coiled hyphal strand. In view of the fact that direct cell penetration by the fungus does not occur, the mycelium evidently gained access to the tracheal vessel through the pits, though this point has not been verified.

The continued progress of the fungus also is frequently impeded by the large clusters of druses present in the tomato leaf.

SUMMARY

The mycelium of *Septoria lycopersici* consists of a hyaline, thin-wall type, and a brown, thick-wall type.

The mode of pycnidial formation is symphyogenous. The pycnidial cavity first forms by a schizogenous process, but, prior to pycnospore formation, a lysigenous action also is involved.

Ostiole formation occurs by tensional strain of the pycnidial wall on the leaf epidermis, and by a pressure of the pycnospores on the pycnidial wall. A lysigenous action also may occur but it was not observed.

Pycnospore formation occurs by basipetal elongation of the sporogenous cells without intervening conidiophores. The pycnospore is separated from the sporogenous cell by a basal constriction.

Pycnospore germination occurs by the formation of a lateral germ tube from the inner cells, and by elongation of the apical cells.

Infection penetration is stomatal. The mycelium is intercellular, and cell penetration is effected by means of haustoria.

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A PHYTOPHTHORA DISEASE OF PEACH SEEDLINGS[1]

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On June 6, 1929, a nurseryman at Bentonville, Arkansas, called the writer's attention to a stem canker and seedling wilt of peach trees in the nursery rows. Nonseptate mycelium was observed in the diseased tissues and an organism, subsequently identified as *Phytophthora cactorum* (L. and C.) Schröter, was isolated readily in pure culture from the diseased seedlings.

Smith (6), in 1915, and subsequently, Smith and Smith (7), described a dying back and gumming of peach and other deciduous nursery stock in California caused by *Pythiacystis citrophthora* and fungi of the *Phytophthora cactorum* group. In 1931 Arnaud and Arnaud (1, p. 1225) observed a wilting of peach trees growing in a greenhouse at Versailles, France. They found a species of Phytophthora present in most of the specimens they examined.

The disease under discussion differs in several respects from these previously described ones. In Arkansas the peach seedlings are attacked and destroyed soon after they appear above ground in the spring. In California the disease apparently has not been observed on seedlings in the spring but a similar malady has been observed on budded nursery stock. The nursery stock is infected in the late fall or winter either before the trees are dug or while they are heeled in. In France the disease developed on grafted trees.

SYMPTOMS

The first symptom of the disease is the appearance of a small, light brown lesion on the stem of the young tree, well above the soil line. The lesion enlarges rather rapidly, generally encircling the stem and develops into a brown, water-soaked canker from 2 to 10 cm. long (Fig. 1, A), which may extend downward to the soil line. The tissues of the affected region are sunken and frequently there is a profuse flow of gum through fissures (Fig. 1, B) in the epidermis.

In the early stages of the disease the leaves appear normal, but as the canker encircles the stem the terminal leaves develop a red discoloration and fail to unfold properly. These symptoms appear only on the terminal leaves.

The root system remains healthy during the early stages of the disease and the root discoloration that develops after the top dies is a secondary effect. The disease is distinctly a disease of the aerial parts.

[1] Cooperative investigations between the Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture and the Arkansas Agricultural Experiment Station.

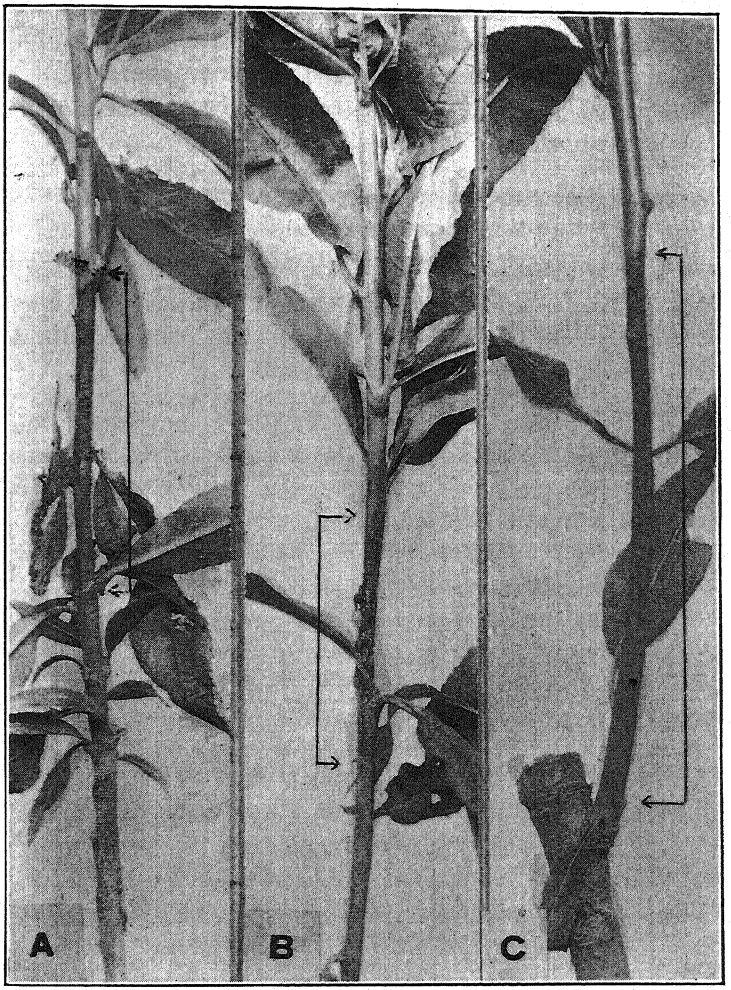


FIG. 1. A. Phytophthora canker on peach seedling. B. Another specimen showing gum oozing from necrotic region. C. Phytophthora canker on a budded tree. The bud was inserted in the fall of 1928 and the shoot was infected after growth started in 1929. All three specimens collected at Bentonville, Arkansas, June 8, 1929. Limits of cankers indicated by brackets.

A few cases have been observed in which the shoot developing from a bud that had been inserted in the fall was infected the following spring (Fig. 1, C). The symptoms on the shoots of the fall-budded trees are the same as those on the seedlings, except that there is a tendency for the shoots to fall over when the final stage of the disease is reached. The infected seedlings do not fall over, as they are supported by adjacent healthy seedlings until the lesions dry out and the tissues become stiff.

Although the majority of the infections seen by the writer developed on the main stem of the seedlings, several cases were observed in 1930 where the initial symptoms appeared on the young branches and none on the main stem. The causal organism was isolated from these cankers as readily as from those on main stems.

THE INFLUENCE OF WEATHER CONDITIONS ON THE OCCURRENCE OF THE DISEASE

It is a well established fact that the prevalence and destructiveness of phytophthora diseases are closely correlated with excessive amounts of rainfall and numerous periods of cloudy weather. The phytophthora disease of peach seedlings is no exception to this general rule.

The weather records (9) for the Bentonville, Arkansas, station (Table 1)

TABLE 1.—*Total precipitation and number of days with .01 inch or more precipitation during the months of April, May and June, 1927 to 1934, inclusive, at Bentonville, Arkansas*

Year	Precipitation in inches				Number of days with .01 inch or more precipitation			
	April	May	June	Total for the three months	April	May	June	Total for the three months
1927	9.50	4.38	7.47	21.35	16	10	12	38
1928	9.85	6.62	13.81	30.28	9	11	19	39
1929	7.85	9.93	8.05	25.83	15	16	14	45
1930	2.46	5.82	4.74	13.02	13	13	6	32
1931	4.16	5.42	3.01	12.59	10	9	7	26
1932	2.09	0.90	5.28	8.27	7	4	16	27
1933	6.93	9.62	0.14	16.69	10	14	1	25
1934	2.07	2.97	2.53	7.57	7	9	6	22

show excessive amounts of precipitation and numerous rainy days for the months of April, May and June, 1927 to 1929 inclusive and a decrease in the subsequent years. The outbreak of the disease under discussion in this paper began in 1927 and continued until 1930, although it was much reduced in severity in 1930. Since that time the disease has not been observed in northwest Arkansas and it is evident that its prevalence is closely correlated with periods of excessive precipitation and rainy weather. The data for

1928 are particularly interesting, for it was then that approximately 50,000 young plants were destroyed in one nursery.

ISOLATION AND DEMONSTRATION OF THE PATHOGENICITY OF THE CAUSAL AGENT

Pure cultures of a species of Phytophthora were readily secured from peach seedlings by aseptic removal of fragments of the inner tissues of young cankers and placing them in Petri dishes of solid corn-meal agar.

The first series of experiments to demonstrate the pathogenicity of the fungus isolated from cankers on peach seedlings was carried out at various intervals from July 18 to September 16, 1929. In these experiments small pieces of agar bearing mycelium were inserted in slits made with a flamed scalpel in the epidermis of succulent branches of the seedlings. No inoculum was inserted in the slits used as checks and all the branches were covered with frequently moistened sterile cotton. Previous to making the slits all the branches used were thoroughly swabbed with bichloride of mercury and then washed with sterile water.

The inoculum was inserted in 47 branches and cankers (Fig. 2, A) and developed on 28 branches. The first symptoms appeared 3 to 5 days after the inoculations were made; necrotic lesions 2.0 to 5.0 cm. long eventually developed on the shoots, but the parts above the lesions did not wilt. The fungus was reisolated from a number of these lesions. The noninoculated, control slits, 22 in number, healed normally.

In the second series of inoculation experiments the inoculum was inserted in slits made approximately 1 inch above the soil line on the main stem of 4 peach seedlings on July 18 and 2 seedlings on July 22, 1929. Cankers (Fig. 2, B) developed on the stems of 5 of these seedling trees above the slits, but the trees did not wilt. The noninoculated slits on 5 additional seedling trees, used as controls, healed normally.

The inoculation experiments performed in 1929 demonstrated that the fungus was pathogenic when introduced into the tissues through wounds. Cankers formed about the site of the inoculation in many cases, but the wilting so typical of the disease in the field did not develop following the inoculations.

In 1930 it was found that the fungus would grow on moistened bran; and on March 17, 1931, bran, previously inoculated with a culture reisolated from a canker produced in the 1929 experiments, was mixed with sand and placed over the soil of a pot containing 2 seedling peach trees growing in a greenhouse. One of the seedlings was a year old but was still rather succulent, while the other seedling was just a few weeks old. A mixture of sterile bran and sand was placed on the soil of another pot containing 2

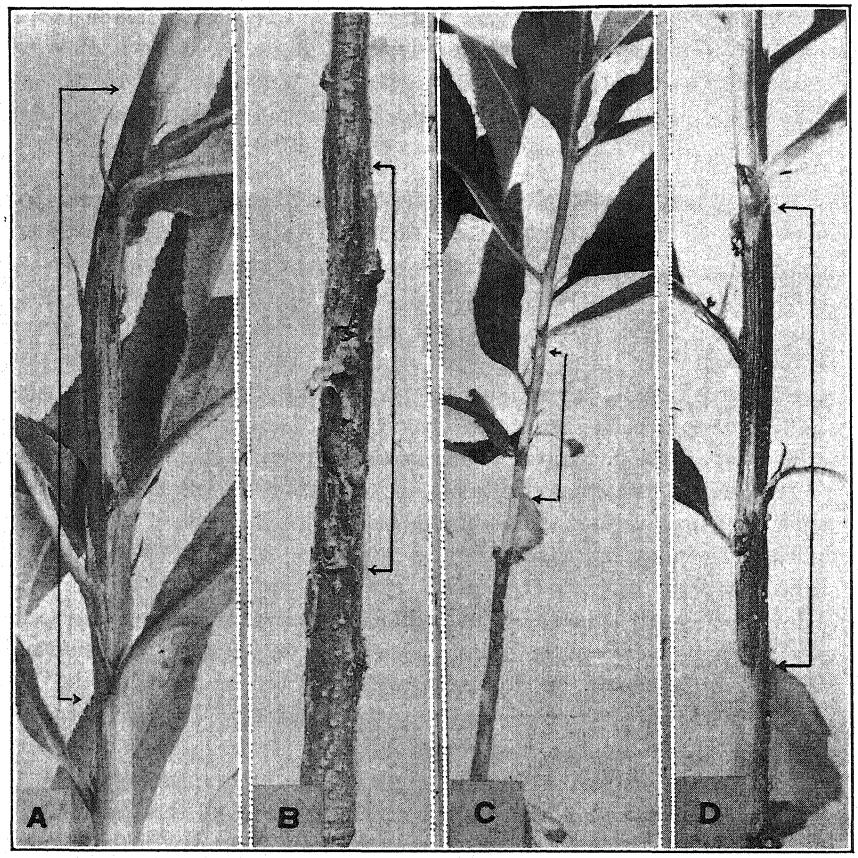


FIG. 2. A. Canker produced by inserting mycelium in a slit made in the epidermis of a succulent shoot of a peach seedling. Inoculated September 16 and photographed September 24, 1929. B. Canker produced by inserting mycelium in a non-succulent stem of peach seedling. Inoculated July 18 and photographed August 26, 1929. C. Canker produced by placing mixture of inoculated bran and sand around young seedlings on April 16, 1931. Photographed April 20, 1931. D. Portion of C enlarged to show invaded region. Limits of cankers indicated by brackets.

small seedling trees to serve as checks. The 2 pots were placed in an inoculation chamber for 24 hours and then returned to a greenhouse bench.

Water-soaked streaks were observed March 19 on the stem of the younger seedling in the pot covered with inoculated bran, and the stem and leaves were slightly flaccid. The seedlings of the same age in the control pot were turgid. At 8 A. M. the following day the infected seedling was turgid again, but 2 leaves at the apex had collapsed, and by 10 : 30 A. M. the seedling had wilted. The partial recovery during the night is of interest because, in

1929, during periods of rainy weather, many seedlings in the nursery rows were observed to be girdled but the tops remained turgid until the advent of clear weather, when they wilted rapidly.

The succulent year-old seedling showed no pathogenic symptoms until March 21, when a water-soaked spot appeared on the stem 1.0 cm. above the layer of bran and soil. Three days later the lesion was 9.0 cm. long and had girdled the stem. The tree wilted subsequently and was dead on March 31. Pure cultures of the organism were isolated from this plant. The 2 control seedlings remained healthy.

On April 16 a flat containing 22 peach seedlings was inoculated by covering the soil with a mixture of inoculated bran and sand. The fungus used in this experiment had been isolated from the year-old seedling in the experiment of March 17. The soil of a pot containing 10 peach seedlings was covered with a mixture of sterile bran and sand to serve as a control. The flat and the pot were placed in a saturated atmosphere for 48 hours and then returned to a greenhouse bench.

Typical cankers (Fig. 2, C and D) developed on the stems of 5 of the seedlings within 4 days and subsequently 3 more seedlings became infected. The leaves of the infected seedlings developed the reddish color and peculiar folded condition noted in the field, but wilting did not develop. The control seedlings remained healthy.

The 1931 experiments demonstrated that the fungus, reisolated in 1929 from inoculated plants, could infect noninjured seedlings with the production of symptoms identical to those observed in the nursery rows, although wilting and subsequent death of the seedlings was reproduced experimentally in only 2 instances.

DESCRIPTION OF THE CAUSAL ORGANISM

The mycelium of the fungus consists of long, profusely branched filaments devoid of cross walls except when old, and ranging in diameter from 2 to 10 microns. In young cultures the hyphae are full of yellowish, granular protoplasm, which flows rapidly through the filaments. As the cultures age the older filaments lose most of their contents and appear as hyaline, empty tubes.

The sporangia are produced sympodially on slender branches (sporangiophores) of the stout hyphae. They show considerable variation in size and shape but generally are ovate in form, measuring 30 to 45 μ long and 24 to 36 μ in diameter. Each sporangium has a small papilla and the internal contents are densely granular. On repeated occasions they have been observed to germinate to form a germ tube that, in turn, may give rise to secondary sporangia, but efforts to induce zoospore production have not been successful.

Antheridia, oogonia, and oospores develop profusely on corn-meal agar and Lima-bean agar, frequently appearing within 48 hours when vigorously growing cultures are used. The oogonia are subspherical, 32 to 38 μ in diameter, and hyaline to pale yellow. The antheridia, which vary in shape from curved tubes to spherical bodies, are predominately paragynous (of a total of 204 antheridia examined only 2 appeared to be amphigynous).

The oospores are thick-walled, 25–30 μ in diameter, yellowish, and are borne within the oogonia.

The organism grows readily on various media, such as corn-meal, Lima-bean, malt-extract and dextrose agars and has also been cultured in flasks of steamed bran for use in inoculation experiments. The amount of aerial mycelium produced varied with the kind of medium and was more abundant on Lima-bean and corn-meal agars than on dextrose and malt-extract agars. While the amount of growth and number of both sporangia and oogonia vary with kind of medium used, the length of time during which any particular transfer had been maintained in artificial culture also is an important factor. Cultures that ceased to produce oogonia could be induced to form them again by inoculating apples with the culture and reisolating from the apple tissue. The fresh isolation would frequently produce oogonia within 48 hours. The organism grew slowly at 5° C., vigorously at 21° to 26°, but was inhibited at 32°. One isolation held at 32° for 10 days and then returned to 21° made little growth at the lower temperature, while another isolation was killed by exposure at 32°.

The hydrogen-ion requirements of the organism were tested on a solid synthetic medium[2] to which soluble starch had been added as a source of carbohydrates. On this medium at 24° C. the organism grew in a range from pH 4.0 to pH 9.0.

TAXONOMY

As the result of the comprehensive studies of the genus Phytophthora initiated by Rosenbaum (5) in 1917 and subsequently carried on by Leonian (3), Leonian and Geer (4), and more recently by Tucker (8), it is possible to identify a new isolation of Phytophthora with some degree of certainty.

Probably the most outstanding characteristic of the fungus isolated from peach seedlings is the prompt and profuse production, in culture, of oogonia with paragynous antheridia. This feature limits the species to be considered to *Phytophthora cactorum* and *P. syringae,* for these are the only species that have predominately paragynous antheridia and that produce oogonia promptly and abundantly in culture. Some investigators might include *P. citricola* and *P. hibernalis,* but the first mentioned form is now

[2] Monobasic ammonium phosphate 1.0 g., potassium chloride 0.2 g., magnesium sulphate 0.2 g., soluble starch 20 g., agar 25 g., distilled water 1000 ml.

considered to be synonymous with *P. cactorum,* and *P. hibernalis* with *P. syringae.*

Phytophthora cactorum and *P. syringae* are closely related forms but, as Tucker (8) and Chester (2) have pointed out, *P. syringae* does not grow in culture at 25° C., while *P. cactorum* thrives luxuriantly at this temperature and is not inhibited until a temperature of 32° is reached. There are corresponding differences in regard to the hydrogen-ion concentration, the growth range for *P. syringae* being pH 3.5 to 7.5, and for *P. cactorum* pH 3.0 to 10.0. According to Tucker (8) the papillae of *P. syringae* sporangia are flattened, while those of *P. cactorum* protrude and often are inconspicuous. Sporangia from corn-meal agar cultures of the peach-seedling organism had small protruding papillae of 1.5–2.0 μ in length.

The peach-seedling fungus in culture, as mentioned previously, is marked by the profuse production of oogonia with paragynous antheridia. The sporangia bear protruding papillae, the organism grows vigorously at 26° C. and not at 32°, and its hydrogen-ion range is from pH 4.0 to at least pH 9.0. Since these are characteristics of *Phytophthora cactorum,* it is concluded that the organism isolated from the stem cankers on peach seedlings is this species.

PATHOLOGICAL HISTOLOGY

Sections were prepared from the cankers produced in the inoculation experiments of March 17, and April 16, 1931. This material was used in preference to that from the nurseries, as the latter was found unsatisfactory for histological work because of adhering soil particles.

The sections show that the fungus affects all the tissues from the epidermis to the cambium. The cells of the epidermis and the parenchyma beneath it collapse into a dark colored mass. The mycelium of the fungus was observed among the distorted cells of the cortical parenchyma but no oospores were observed. Fragments of older cankers were dissected to ascertain whether oospores developed in the tissues as the disease progressed, but they were not found even in this older material.

The cells of the cortical fibers are discolored but retain their shape. Their presence, however, offers no barrier to the progress of the fungus, as the cells of the phloem and cambium were completely destroyed in most of the sections. The space formerly occupied by these tissues is filled with a series of gum pockets and only fragments of the former cellular structure are visible. The mycelium of the fungus has been observed along the boundaries of the gum pockets and in a few instances fragments of the mycelium were detected in the gum pockets. The youngest cells of the xylem adjacent to the cambium are discolored but, on the whole, the xylem and pith appear uninjured by the fungus.

CONTROL OF THE DISEASE

The only known practical control measure is to avoid growing seedlings in poorly drained areas. This procedure does not eliminate the possibility of the disease appearing in the nursery if environmental conditions are exceptionally favorable, but a number of nurserymen have told the writer that they have reduced the loss to a negligible figure by selecting well-drained sites for their seedling nurseries.

The low value of an individual seedling in a planting of 50,000 to 100,000 seedlings, the extreme susceptibility of the peach to spray injury, and the variation in prevalence of the disease in various parts of a nursery are factors that make the problem of disease control in the nursery a very difficult one.

In 1930 the writer attempted to control the phytophthora disease of peach seedlings by applying such materials as aluminum sulphate, zinc sulphate, flowers of sulphur, ground flour of sulphur, and a monosulphide of sulphur to the ground along the nursery rows close to the trees. These materials are relatively inexpensive, and by applying them to the ground instead of spraying them on the trees the risk of injury to the foliage was eliminated. When the results were recorded on June 6, 1930, it was found that, while the non-treated control plot showed 5 per cent of the seedlings affected, the disease was present in the treated plots in varying degrees, ranging from 1 to 26 per cent. This irregular distribution of the disease in plots selected at random completely obscured the recognition of any beneficial results that might have been secured by the various treatments.

The absence of the disease since 1930 has prevented further experiments on this phase of the problem.

SUMMARY

A disease of peach seedlings caused by *Phytophthora cactorum* and characterized by the formation of a canker on the succulent stems, a wilting of the parts above ground and the ultimate death of the young trees, has been observed in northwest Arkansas.

The fungus attacks the seedlings soon after they emerge above ground in the spring.

The symptoms of the disease are described in some detail. The disease is confined to the aerial parts, the root system remaining healthy until the death of the aerial parts ensues.

The occurrence of the disease is favored by abundant rainfall and cloudy weather during the early part of the growing season.

Pure cultures of an organism identified as *Phytophthora cactorum* were readily secured from the diseased tissues. The characters of the fungus in culture are briefly discussed. The pathogenicity of the fungus was proved

by a series of inoculation experiments culminating in the production of typical wilt of the experimental plants growing through a mixture of sand and inoculated bran.

A study of the cankers shows that the effects of the fungus are confined to the cortex and cambium. Gum pockets are formed in the region of the phloem, while the outer parenchyma tissues collapse into a dark discolored mass resting on the cortical fibers.

The only practical control measure known is to grow the seedlings in well-drained sites.

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SUMMARY OF INVESTIGATIONS WITH USTILAGO STRIAEFORMIS PARASITIZING SOME COMMON GRASSES

W. H. DAVIS

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Some of our common grasses are parasitized by a leaf smut (*Ustilago striaeformis* (Westd.) Niessl). This species is an assemblage of species due to the fact that previous investigators have classified the organism according to morphological characters. Mycologists were unable to find consistently varying morphological spore characters and symptoms of the smut in the various hosts, so they assigned the smut in each grass host to the same species. Furthermore, they were unable to consider the biology of the fungus because they did not have the spore germination under control for inoculation experiments and life-history studies. The conditions of spore germination, however, have been solved (2), the life-history described (3), and two physiologic forms reported (4), one in timothy, *Phleum pratense* L., and another in redtop, *Agrostis palustris* Huds. The biology, however, of the American forms of the smut in orchard grass, *Dactylis glomerata* L., Kentucky bluegrass, *Poa pratensis* L., and annual bluegrass, *P. annua* L., has remained unsolved.

Liro (5: 317, 377, 380) believed that the original specimen of *Ustilago striaeformis* in *Holcus lanatus* L. was none other than the uredo form of *Puccinia dispersa* Eriks. and Henn., and the host was *Triticum repens* instead of *Holcus lanatus.* He does not, however, wish to consider his examination of *exsiccati* as final, since a sufficient number of distributed specimens was not available for his examination. Nevertheless, Liro's researches cause one to doubt the advisability of assigning these smuts in some of our agricultural grasses to the one species, *U. striaeformis,* and they have shown need for further investigation.

Liro (5: 372, 379, 392) reported leaf smut on *Dactylis glomerata* in Europe as *Ustilago salveii* Berk. and Broom. His investigations regarding *U. salveii* showed that it is a distinct morphological species possessing spores smaller than those of the typical *U. striaeformis;* 8×12 as compared with 10×15 μ. Furthermore, *U. salveii* was biologically fixed to its host plant, since it neither infected species of Phleum, Festuca, Poa, nor Agrostis, growing in the same garden with infected *Dactylis glomerata,* nor did it infect inoculated species of Festuca, Anthoxanthum, Aira, Arrhenatherum, Calamagrostis, *Agrostis canna* and *A. vulgaris, Avena elatior, Alopecurus* and Milium.

It is noteworthy that Liro found the spores germinating in nature during August and no after-ripening period of 254 days was reported,

as occurs in our American species (2). Besides, he did not inoculate timothy, redtop, Kentucky blue, and annual bluegrasses, thus leaving the biologic relation of the smut in these grasses and the striped smut in our orchard grass unsolved. He also raised the question as to whether the smut in the American species of orchard grass is a physiological form of *U. striaeformis, U. salveii* or a definite species as yet unnamed.

No published investigations regarding the biology of *Ustilago striaeformis* in *Poa pratensis* and in *P. annua* are known to the writer and it is obvious that these should be investigated. With the above information at hand, the writer undertook by investigation to answer the following questions:

1. Is *U. striaeformis* a single species or an assemblage of several species of smut fungi that parasitize many of our grasses?

2. What Latin binomial should be assigned to the leaf smut of orchard grass? Should it be referred to *U. salveii, U. striaeformis,* or a new species of Ustilago?

3. Are there physiologic forms of *U. striaeformis* on *Dactylis glomerata, Poa partensis,* and *P. annua?*

METHODS AND MATERIALS

The methods employed were essentially those already described in a previous report (4). The materials also were assembled, the spores stored for ripening, and the grass seeds cultured according to the method previously employed (4). There were, however, some additions and variations that should be presented.

Dactylis glomerata, infected with the leaf smut, was difficult to locate. From 1921 to 1933, the writer has made field observations in Iowa, Wisconsin, Illinois, New York, Pennsylvania, and in each of the New England States. New York is the only State in which this smut could be located as parasitizing this grass. Furthermore, specimens of this smut have been solicited repeatedly from pathologists, but not one new station has been located by them and reported. So the 3 New York State stations located at Ithaca, Loon Lake, and Elmira, respectively, are the only natural stations known to the writer at this time. *Dactylis glomerata* plants bearing smutted leaves were removed from one of these stations and transplanted in the pathological garden at the Massachusetts State College. Furthermore, spore materials were collected from each of these 3 stations and the spores properly stored for ripening. When tests showed a 35 per cent germination or better, the spores were employed for inoculating seedlings of orchard grass. Plants infected by artificial inoculation were transplanted and retained in the pathological garden for 5 years.

They furnished a sufficient supply of spore materials each year, since the fungus was perennial within them.

Inoculated plants of *Dactylis glomerata* often were retained in the plots for 2 years before the first symptoms of the smut were observed. The symptoms generally showed best in matured plants during the early spring and the autumn.

Seed of *Dactylis glomerata* was obtained from two reliable seedsmen, but the agronomic strains were unknown. Five collections also were obtained from matured field plants under cultural conditions in Massachusetts. Only two of these, however, were used in the inoculating experiments, since no strain seemed immune from this smut fungus.

Poa pratensis infected with leaf smut was located in Wisconsin and New England. Amherst, Massachusetts and Windsor, Connecticut, were the 2 best stations for collecting spore materials. At times, 30 per cent of the plants at these stations was infected and 85 per cent of the infected seedlings often killed under adverse host conditions that afterwards developed. Spore materials were collected from infected hosts with partly matured panicles, from immature plants, and from seedlings. The spore materials were incubated and the spores ripened within periods varying from 110 to 250 days. The percentage of germination, however, was rather low, varying from 35 to 60. The seedlings that were inoculated had been cultured from seeds purchased of 2 reliable seedsmen located in Massachusetts.

Smutted plants of *Poa annua* were more difficult to locate than those of *P. pratensis* and *Dactylis glomerata*. For definite determination of the hosts, floral parts removed from infected plants were classified by A. S. Hitchcock and his associates. Spores collected in the spring germinated in late autumn, 1929. The ripening period varied from 94 to 128 days. Only 2 lots of these spores were collected on account of the tedious work of locating a sufficient quantity of spore materials for inoculating purposes. One lot germinated 95 per cent, following storage for 150 days under proper conditions. Thus an attempt was made to use spore materials from properly classified hosts and from as many stations as possible so as to include any physiologic race that might be confined to one locality. Furthermore, the grass seeds were unassorted, so as to include both susceptible and resistant strains of hosts, provided they existed.

INOCULATIONS

The inoculations were begun in 1928 and performed during different seasons of each year until 1934, when the experimental plots were destroyed by unscrupulous individuals. The results of the inoculation work with spores from *Dactylis glomerata, Agrostis alba, Phleum pratense, Poa*

pratensis and *P. annua* had, however, been observed and recorded. But those with sweet vernal, *Anthoxanthum odoratum* L., and other experiments were destroyed.

Only 2 grass hosts were inoculated in any one series so that both reciprocal inoculation and inoculations within the hosts species could be conveniently performed. Lots of *Dactylis glomerata* and *Phleum pratense* seedlings were each inoculated with germinated spores from both, a total of 27 times; *D. glomerata* and *Agrostis alba* were similarly inoculated in 9 series *D. glomerata* and *Poa pratensis,* 9 times; *D. glomerata* and *Poa annua* 4 times.

The observations showed smutted orchard grass plants in each series, except one, in which smut spores from *Dactylis glomerata* were employed. All inoculation of this grass with spores from other hosts failed to produce infections. The percentage of infected plants varied from 2 to 20 for those inoculated, and the incubation period varied from 8 weeks to 2 years before rugae of smut spores were observed in orchard grass leaves. These results showed that the smut in orchard grass is biologically differentiated from that in *Phleum pratense, Agrostis alba, Poa pratensis* and *P. annua.*

To determine the biology of *Ustilago striaeformis* in *Poa pratensis,* a plan similar to that already described for *Dactylis glomerata* was followed, but germinated spores of the *P. pratensis* strain were employed as the main inoculum. The reciprocal inoculations and inoculations within the host species follow: *P. pratensis* and *Phleum pratense,* 7 series; *Poa pratensis* and *Agrostis alba,* 8 series; *Poa pratensis* and *D. glomerata,* 9 series; *Poa pratensis* and *P. annua,* 3 series. The smut striae showed distinctly in the seedlings of *P. pratensis* only when inoculated with spores from that grass. All other inoculated plants remained smut-free. The percentage of infected plants varied from 0, in one case, to 60, with an average of 22 per cent for all other suscepts inoculated. These results also show that the *P. pratensis* strain is biologically different from those of timothy, redtop, orchard grass and annual bluegrass.

Smut spores of the *Poa annua* strain were similarly used for determining its biological relations to timothy, redtop, orchard grass and June grass strains. From the 2 lots of ripened spores, 3 series of inoculations of each host species and reciprocals were made. The percentages of infected *P. annua* plants for the series varied from 2 to 8 and all these were within the host species of *P. annua* that had been inoculated with spores from this grass and no infection showed elsewhere.

These results showed that the *Poa annua* strain of smut differs biologically from the *Phleum pratense, Agrostis alba, Dactylis glomerata* and *Poa pratensis* strains. Furthermore, that leaf smut in *P. pratense, A. alba, D. glomerata, Poa pratensis* and *P. annua* is in each instance a physiologic

form peculiar to its specific host, considering the conditions under which the work was performed.

FIELD OBSERVATIONS

From 1920 to 1934, the writer made field observations on *Ustilago striaeformis* and collected it in the following States: Wisconsin, Iowa, Illinois, New York, Ohio, Pennsylvania and the New England States. The observations regarding physiologic forms of the smut in the fields may be summarized:

Infected *Phleum pratense, Agrostis alba,* and *Poa pratensis* have been collected in contact with *Dactylis glomerata,* yet no infected plants of the latter were observed at those stations.

The scarcity of leaf smut in *Dactylis glomerata* would lead one to believe that it is either a physiologic form or a distinct species.

Many smutted plants of *Poa pratensis* have been found growing in contact with *Agrostis alba,* which remained perfectly healthy in that vicinity.

Also, the paucity of smutted plants of *Poa annua* affords some evidence in support of physiologic specialization, or a different species.

These observations present additional evidence of physiologic specialization of the smuts in *Dactylis glomerata, P. pratensis* and *P. annua.*

NOMENCLATURE

The question arises as to whether leaf smut of *Dactylis glomerata* should be referred to *Ustilago salveii, U. striaeformis* with its physiologic form, or to a new species. As previously stated, Liro considered *U. salveii* as a distinct species parasitizing *D. glomerata* cultured in Europe. His classification was based on the facts that the spores were smaller than *U. striaeformis* found in some other hosts and that it was "biologically fixed" to its host.

Experimental evidence presented in these data, shows that the smut on *Dactylis glomerata* in the United States also is biologically fixed to its host. However, the spores are slightly larger than those from other host species examined and considerably broader than those of *Ustilago salveii,* 10×12 μ as compared with 8×12 μ for *U. salveii* and 9×11 μ for the other grass smuts (Table 1). Nevertheless, a constant physiologic basis seems of more value in determining this species than such a variable morphological basis as spore measurements. The measurements were made from spores collected during a 2-year period and after the spores had been subjected to conditions varied by locality, ripening, germinability, desicacation, moisture, season, storage of host, position in the hosts, light, and heat. Since there was such a degree of variability for different lots of spores, biometric studies, already begun, were discontinued and only limits of variation together with arithmetical means were considered (Table 1).

TABLE 1.—*Measurements assigned to spores of some leaf smuts of grasses*

Authority	Hosts	Measurements—*U. striaeformis* (Microns)
Clinton	Grasses	9–14 long; spherical to ellipsoidal.
Davis	Grasses	9 × 11 standard; 8–11 × 9–15, variation.
Liro	Grasses	8–11, limitations for all measurements made.
	Dactylis glomerata	8 × 12. For *Ustilago salveii*, only.
Davis	" "	10 × 12, standard; 7.6–12 × 10.2–15.3, variation.
	Phleum pratense	8.8 × 11.3. Average for 8 collections.
	Agrostis alba	9.3 × 11.4. Average for 10 collections.
	Poa pratensis	8.4 × 10.4. Average for 3 collections during 2 seasons.
	P. annua	8.2 × 10.0. Average for 2 collections during 1 season.

These showed that the spores of *U. striaeformis* in *Dactylis glomerata* were larger than those from the other hosts examined. Furthermore, spores from this grass generally could be identified by the steel gray to smoky hue replacing the shades and tints of brown observed in spores from other grass hosts. Also, spores in some collections were somewhat irregular in outline and had a tendency to collect in a mass or "spore ball" that consisted of several hundred spores. In most respects, this fungus behaved differently from those of other grass hosts; so it seems best to consider this as a separate species, **Ustilago clintoniana,** sp. nov.

Rugae or black pustules of spores located between leaf traces in leaf blades, sheaths and stems of *Dactylis glomerata* cultured in New York State and Massachusetts. The host epidermis is opened and the dusty spores emerge leaving portions of the leaf blades in a shredded condition. Spores often assemble in globose to elongated masses or "spore balls," ellipsoidal and mostly regular in outline; color, a smoky brown; echinulations prominent, about 40 on one facies; spores 10 × 12 μ. Differentiated from other forms of *Ustilago striaeformis* in grass hosts by its larger, darker spores, more regular in outline and tend to cling in spore balls, attached or unattached to host tissue. When they germinate, their germ tubes are longer and the protoplasm collects in the tips. The habitat is not commonly associated with other grass smuts, as it occurs in isolated places. The fungus is biologically fixed to its host under the conditions examined. Type specimens were deposited in the Cryptogamic Herbarium of Massachusetts State College, and the accession number is 3319.

The forms of *Ustilago striaeformis* in *Poa pratensis* and *P. annua* compare favorably with those in *Phleum pratense* and *Agrostis alba* as regards spore color and behavior. Spores from these two hosts, however, were smaller than from *Phleum pratense* and *Agrostis alba* (Table 1) under

the conditions examined. These variations in size were not deemed sufficient to establish a separate species in each case in view of the fact that each was physiologically fixed to its host; they, therefore, will be considered as physiologic forms of *U. striaeformis*. The following classification is suggested for the United States forms:

1. *Ustilago striaeformis* forma *Phlei.*
   Host: *Phleum pratense* L.
2. *Ustilago striaeformis* forma *Agrostidis.*
   Host: *Agrostis palustris* Huds.
3. *Ustilago striaeformis* forma *Poae-pratensis.*
   Host: *Poa pratensis* L.
4. *Ustilago striaeformis* forma *Poae-annuae.*
   Host: *Poa annua* L.
5. *Ustilago clintoniana,* sp. nov.[1]
   Host: *Dactylis glomerata* L.

This classification is not strictly in keeping with Liro's, for he listed a separate species on each host and gave it a Latin binomial consisting of the genus Ustilago with a species significant of the host parasitized or to which it was biologically fixed (5:373). With this in mind, the first four species would then become: *Ustilago phlei-pratensis; U. agrostis-palustris; U. poae-pratensis; U. poae-annuae,* each a *species novum.*

In the future, other hosts may be found for these physiologic forms. Furthermore, undescribed physiologic forms may exist on other hosts, as only a small number of the large host list has been under experimentation.

SUMMARY

A summary of 12 years' observations and experimentation with *Ustilago striaeformis* parasitizing *Phleum pratense, Agrostis alba, Dactylis glomerata, Poa pratensis,* and *P. annua* is presented. These findings may be briefly stated:

The conditions for spore germination have been determined.

The life histories are known; infection is initiated in the seedling.

The genus has been determined; each smut belongs to the genus Ustilago, which was determined by the type of spore germination.

A form of the smut fungus is physiologically fixed to each host examined.

The smut on orchard grass is here considered a distinct species, *Ustilago clintoniana,* sp. nov.

A physiologic form is assigned to each smut parasitizing the other 4 hosts, since the spores bear resemblance to the accepted type form parasitizing timothy and often called "timothy smut" in literature.

[1] Named in honor of Dr. G. P. Clinton who was first to germinate the spores removed from redtop and definitely change *Tilletia debaryana* F. de Wald. (*T. striaeformis* Oud.) to *Ustilago striaeformis* by presenting the proper, accurate, scientific data.

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RESISTANCE OF PINUS SYLVESTRIS TO A GALL-FORMING PERIDERMIUM[1]

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INTRODUCTION

In 1926, York (43) reported that a gall-forming Peridermium was causing considerable injury to Scotch pine, *Pinus sylvestris* L., at the Masonic Summer Camp on Round Lake in the vicinity of Woodgate, New York. It has yet not been definitely determined[3] whether this is a new species of Peridermium, some atypical, autoecious form of *Cronartium quercuum* (Berk) Miyabe (*C. cerebrum*), or one of the western-United States gall rusts. Growing among the heavily infected trees in this Woodgate area are many trees that, although artificially inoculated for 6 successive years, have shown no gall formation. The present investigation deals with this phenomenon of resistance as studied from the standpoints of gross morphology, histology, and physiology.

LITERATURE REVIEW

There is a marked paucity of literature, other than observational accounts, on the resistance of forest trees to fungus disease; in fact, there are few recorded attempts to study the basis of resistance in woody plants of any kind. Most discussions relative to the resistance of plants to the invasions of parasites point out that resistance may have a morphological or a physiological basis or a combination of the two. The formation in the host tissue of a definite layer of cork cells in advance of the invading mycelium has been considered by several investigators to represent an attempt to check the spread of the parasite. Conant (9), in studying the cause of resistance of certain strains of tobacco to *Thielavia basicola* (B.

[1] A thesis submitted to the Graduate School of the University of Pennsylvania in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Botany.

[2] The writer is indebted to Dr. H. H. York for his helpful advice and criticism during the progress of this work.

[3] In the recently published Manual of the Rusts in United States and Canada p. 24, Arthur states "Another rust on *P. sylvestris* . . . recently discovered in northern New York and called 'Woodgate Rust'" is "now referred to *C. quercuum.*"

and Br.) Zopf, recorded that such corky areas between diseased and healthy tissues successfully prevented the spread of the fungus through the plant. Other investigators have stated that the resistant character of cell walls may account for protection against disease. Melander and Craigie (26) found that varieties of Berberis immune from *Puccinia graminis* Pers. were provided with thicker cuticles and with cell walls more resistant to mechanical puncture than were those susceptible to infection. In many instances the products of cell metabolism and their physical and chemical relationships have been considered to have a very direct bearing upon resistance and susceptibility. Dufrénoy (14), from a study of *Blepharospora cambivora* Petri on chestnut, *Phytophthora infestans* (Mont.) de Bary on potato, and *Colletotrichum lindemuthianum* (Sacc. and Magn.) Br. and Cav. on bean, has concluded that the accumulation of phenolic compounds in vacuoles represents a local immunity in the plant. Newton, Lehmann, and Clarke (29) have suggested a relation between the resistance of wheat to *Puccinia graminis tritici* Eriks. and Henn. and the phenolic compounds present in the plants. Cook and his associates (10, 11) have shown that tannin may exert an inhibitory action upon the growth of many species of fungi in culture. A careful biochemical study by Angell, Walker, and Link (4) has recently shown that resistance of onions to onion smudge is definitely related to the presence of protocatechuic acid in the outer pigmented scales. Dickson, Link, and Dickson (12) have found in the case of seedling blight that resistant corn seedlings contained 20 per cent more uronic acids than susceptible seedlings. Ezekiel (16), in an extensive study of resistance of wheat to *Puccinia graminis tritici,* has recorded a correlation between resistance and the growth and branching of germ tubes of the spores in extracts from resistant and susceptible plants. Thomas (36) reports that, although the growth of *Armillaria mellea* (Vahl) Quel. is inhibited by the extractives of the roots of certain trees, there apparently is no relationship to resistance. It is conceivable that the cell sap of certain plants may be uncongenial for fungus growth because of its lack or excess of hydrogen ions. Many experimental attempts have been made to demonstrate such a relationship. Comes (8) has found that a rust-resistant strain of wheat has a greater acidity than susceptible forms. When this same strain, however, is grown under different environmental conditions it becomes susceptible and its cell sap then shows a reduced acidity. On the other hand, many investigators have failed to find any such relationship. Hurd (20), as well as Newton, Lehmann, and Clarke (29), could demonstrate no relation between the pH of cell sap and the resistance of wheat to *Puccinia graminis tritici.* Harris and his coworkers (19) have clearly shown that the osmotic concentration and electrical conductivity of sap expressed from phanerogamic parasites are greater than

those from the host plants. Similar work has been done to determine whether these physical values were any different in resistant and susceptible plants. Newton, Lehmann, and Clarke (29) have been unable to demonstrate any important difference between tissue fluids of wheat plants resistant and susceptible to *Puccinia graminis tritici* as evidenced by osmotic pressure or electrical conductivity.

In addition to the factors considered above, a number of investigators have found that in plants subjected to infection, especially by rusts, there may be a type of reaction referred to as "protoplasmic resistance" or as "hypersensitiveness." This phenomenon was first observed and studied by Ward (37, 38, 39). He noted that in resistant plants hyphae were formed and haustoria penetrated host cells, but that such cells died soon after invasion, and that the hyphae and haustoria degenerated and appeared like experimentally starved mycelium. He attributed this reaction primarily to starvation of an obligate parasite. Marryat (25) studied infection by *Puccinia glumarum* Eriks. & Henn. in both resistant and susceptible strains of wheat. She also considered that resistance was brought about by the death of host tissue resulting in the death of the parasite from starvation. She further suggested that there might be a formation of toxin and antitoxin perhaps mutually destructive. Gibson (18) studied the early growth of various rusts that had infected plants other than their natural hosts. She rejected the starvation hypothesis of Ward, and considered that toxic substances formed by the host cells might be responsible for the failure of the hyphae to form haustoria and for the ultimate death of the mycelium. In natural hosts that were resistant, she found haustoria present, but the mycelium had degenerated after a few days. This phenomenon of hypersensitiveness in wheat infected with rusts also was studied by Stakman (33). He found in susceptible plants a ready adjustment of parasite and host, but an intolerant relationship in resistant plants. The rust hyphae, although forming few haustoria in resistant plants, brought about the death of host cells adjacent to them. They continued to grow for a time and to affect other cells, but soon perished. In some cases haustoria continued to live after the death of the cells that they had penetrated. He, therefore, concluded that the reaction is not due primarily to starvation but to a toxin-antitoxin relationship. Mains (24), investigating infection in strains of corn resistant and susceptible to *Puccinia sorghi* Schw., suggested that the normal symbiosis among cells of the plants is destroyed by the action of the fungus, causing some of them to parasitize others. He considered carbohydrates to play an important rôle in this resistance phenomenon.. Allen (1, 2, 3), in a detailed cytological study of wheat resistant to *Puccinia graminis tritici*, has regarded Ward's theory of starvation as "untenable." She has suggested that an antagonistic relationship obtains

in the cell in that the cell is first killed by toxin produced by the haustorium, which in turn is killed by the products of cell degeneration.

The relation of environmental factors to susceptibility and resistance has received much attention. Eckholt (15) is inclined to consider environmental factors, such as the physical and chemical nature of the soil, soil moisture, and soil temperature, of more importance in infection of wheat with *Puccinia glumarum* than hereditary factors of resistance and susceptibility. In an extensive study of wheat grown in nutrient solutions, Doak (13) has recently found that an excess of potassium or lack of nitrogen increases resistance to *Puccinia triticina* Eriks.

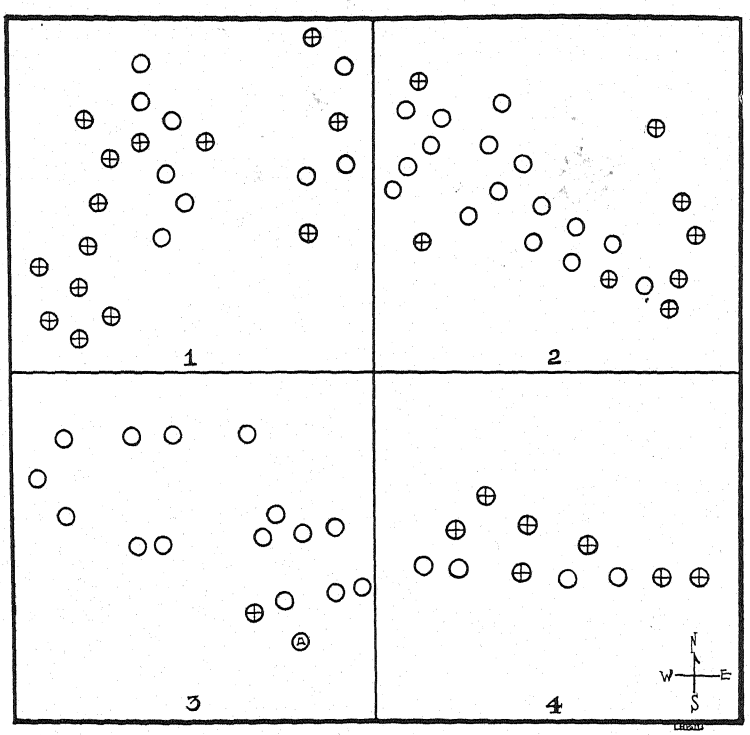


FIG. 1. Diagram of plots of Scotch pine trees. ○, resistant; ⊕, susceptible; A, location of parent tree.

METHODS

Description of plots. In the early summer of 1930, investigations were begun on 4 plots of *Pinus sylvestris* at Woodgate, N. Y. (Fig. 1). Some of the trees in these plots had been inoculated previously by H. H. York. In plot 1, resistant trees of varying age and height are growing near susceptible trees of about the same age and height. Plot 2 consists of

a few trees 8 to 10 years old. About half of these are resistant and half susceptible. In plot 3, there are several trees 10 to 12 years old, all of which are apparently seedlings from one tree, the position of which is indicated by the letter A. This tree, although exposed to natural infection, never developed galls, but succumbed to Armillaria infection. Every tree in this plot except one shows marked resistance. Plot 4 consists of numerous resistant trees about 20 years old growing near groups of susceptible trees.

Inoculations. For 3 consecutive seasons, resistant and susceptible trees have been artificially inoculated. Quantities of galls with mature aecia have been collected in June and masses of aeciospores scraped from them. The terminal portions of branches with the current season's growth were heavily painted with suspensions of these spores in water. The iceless refrigerator described by York[4] was used as a protection about these inoculated branches. This chamber was removed at the end of 48 hours.

Collections have been made from such inoculated branches at intervals varying from 48 hours to 6 years after inoculation. Similar collections have been made from noninoculated trees growing at a distance of 15 miles from the site of the plots, and also a few collections from healthy trees at the Arnold Arboretum, Jamaica Plain, Massachusetts.

Preparation of Tissues. In the preparation of tissues for sectioning several killing and fixing solutions have been used. Formalin alcohol (4 parts formalin to 100 parts 70 per cent alcohol), Carnoy's solution, and Schaffner's chromo-acetic-acid solution have given particular success. Bouin's solution, picro-formol, Fleming's fluid, and Conant's solution also have been used with success.

Some of the tissues were imbedded in nitrocellulose according to the technique described by Jeffrey (21) and Wetmore (41), excellent sections being obtained by this method. In order to obtain serial sections, however, many tissues were imbedded in paraffin by the butyl-alcohol method described by Zirkle (45). This procedure has been found invaluable for the cutting of 1- and 2-year-old stems. Sections, whether cut on the rotary or sliding microtome, were cut from 8 to 15 microns thick. Transverse sections have been used largely, but in many instances radial and tangential sections also.

Staining. The sections were stained with several combination stains. Those intended primarily for the study of normal or abnormal histology usually were stained with the safranin-haematoxylin combination or Fleming's triple stain (6). When it was desired to study the sections for the presence of mycelium and to trace this mycelium through and between cell walls, the combination of orseillin and anilin blue described by Strasburger (35) was used with excellent results.

[4] York, H. H. Celluloid "iceless refrigerator" and some results from field inoculations with the aeciospores of Woodgate rust (*Peridermium* sp.). In manuscript.

Microchemical tests. In connection with the histological study of sections prepared by imbedding, a series of microchemical tests was made on sections freshly cut on the ether-freezing microtome. In most cases the tissues were sectioned a few minutes after they were removed from the tree. Starch, tannin, fats, resin, gums, cellulose, suberin, cutin, lignin, and pectin were identified by means of tests outlined by Eckerson[5] or Stevens (34).

Physiological studies. Quantitative chemical analyses have been made of young twigs from both resistant and susceptible trees with regard to tannin, calcium, and potassium content. Analyses of various samples of soil collected at the base of both resistant and susceptible trees have been made with reference to the quantitative determination of calcium and potassium.

Juices expressed from the twigs of resistant and susceptible trees by pulverizing the ground twigs with liquid air and subjecting this frozen mass to a pressure of 200 pounds per square inch for 10 minutes have been used for determination of osmotic pressure and hydrogen-ion concentration.

An attempt has been made to reproduce in the resistant twigs the pathological changes found following infection. Extractives prepared from galls and from germinating spores were introduced into the tissues through small capillary tubes and also by hypodermic injection. Oxalic acid has been used in the same way, several investigators having reported a relationship of this acid to the pathogenicity of fungi (5, 22).

FIELD OBSERVATIONS OF HOST REACTIONS

In their response to infection the inoculated trees have shown many different reactions ranging from highly susceptible to resistant. For the purposes of this discussion, however, 3 principal types of reaction may be distinguished. Type A occurs only in susceptible trees. During the first growing season there may be slight evidence of infection other than small brownish spots followed by minute swellings. The following spring, however, marked hypertrophies develop and typical galls soon form (Fig. 2, A). In the reaction here designated as type B, there is often definite evidence of infection a few months after inoculation. Isolated discolored areas become confluent and for several inches along the twigs there will be reddish-brown discolorations. During the following growing season an excessive splitting of bark takes place on these twigs, sometimes accompanied by a slight resinosis (Fig. 2, B). Occasionally a few abortive or atypical galls may develop from such areas. In type C reaction numerous small spots may be noted a few weeks after inoculation (Fig. 2, C). These are often greenish brown or slightly reddish in the center, sometimes sur-

[5] Eckerson, S. H. Microchemistry. University of Wisconsin. (Mimeographed).

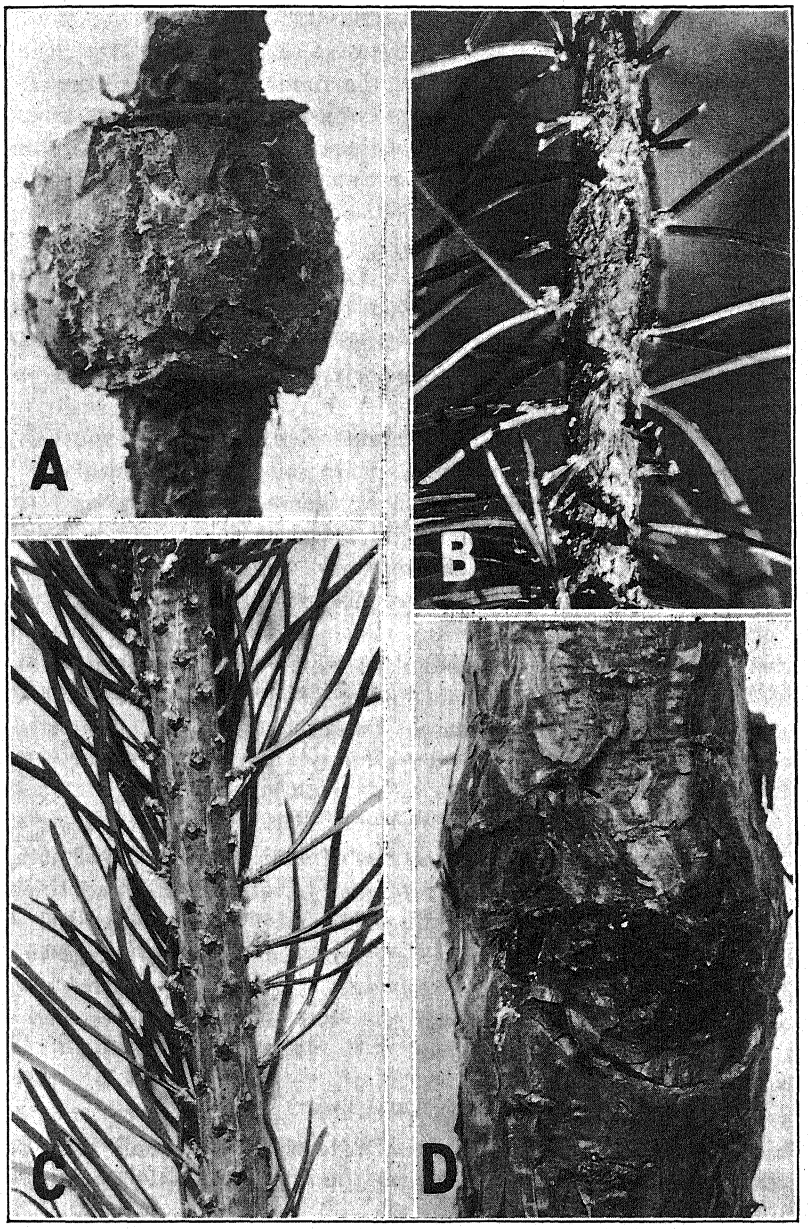


FIG. 2. A. Twig of susceptible Scotch pine 3 years after inoculation showing gall typical of type A reaction. B. Partially resistant twig 1 year after inoculation showing cracked bark characteristic of type B reaction. C. Resistant twig 3 months after inoculation showing spotted areas commonly found in type C reaction. D. Resistant twig 3 years after inoculation showing exfoliation of bark often found in type C reaction. Photographs by H. H. York.

rounded by a lighter chlorotic halo. Similar infection spots have been found developing on various resistant species of pine artificially inoculated with this rust (28, p. 199). During the following growing season, it is not uncommon to find a few swellings developed on these twigs as though galls were to be produced. These swellings rarely increase in size during the third year, but, through the growth of the surrounding tissue, they gradually become indistinct. During the third or fourth year, there can be noted at the position of these swellings a marked exfoliation of bark and sometimes a slight atrophy (Fig. 2, D).

It should be pointed out here that the different reactions described above are not always definitely segregated to certain trees. For instance, types B and C both may be found associated with type A on susceptible trees. Type C, and occasionally type A, may be associated with type B on what we may call partially resistant trees. However, type C only is found on the truly resistant trees. This point will be considered later in connection with the interpretation of the resistant reactions.

Perhaps a brief explanation should be given in connection with the terms "resistant," "partially resistant," and "susceptible," as used above. Gall formation is used as a criterion of susceptibility, since it is only those trees producing galls that show any visible signs of injury. Those trees may be considered partially resistant in which the majority of infection centers are prevented from forming galls. The trees that, although they become infected, are able in some way to check all infection centers and produce no galls can be considered resistant.

HISTOLOGICAL STUDIES

A comparative study of serial sections of noninfected twigs of both susceptible and resistant trees with reference to size of cells and thickness of cell walls has shown no outstanding difference. A microchemical study made of such twigs has shown no significant difference in regard to food storage or chemical character of cell walls. There is considerable variation in the amounts of tannin and fat, but these substances also vary widely among twigs of the same tree.

The Normal Twig

This brief description of the normal twig will include only those structures that have a bearing on penetration and spread of the fungus. The description applies equally to resistant and susceptible twigs during the period when they are subject to infection.

The single layer of epidermal cells is covered over by a cuticle averaging 3 μ in thickness. The cortex consists of 10 to 20 cell layers, the outer 4 or 5 of which rarely store starch during the season when infection might

occur, although the other cell layers may store large quantities. Starch, often in association with fats and resins, also may be stored in the pith, medullary and xylem rays, and phloem parenchyma. Tannin, for the most part, is confined to isolated cells or groups of cells in the cortex, phloem parenchyma, and pith. It also may be found in and near the cambium and in the vicinity of leaf traces. A well-developed periderm may be distinguished during the first growing season. The phellem cells give tests for suberin when tested with ammoniacal gentian violet. A continuous layer of sclerenchyma forms directly internal to the periderm. These cells show a positive lignin reaction. Usually the 3 layers of xylem elements nearest the cambium are free from lignin.

Penetration

The usual method of entrance of the fungus into the host is through the young twigs (44). The germ tube of the spore penetrates the cuticle of susceptible and resistant trees with apparently the same ease (Fig. 3, F and G). No evidence has been secured to show whether the method of penetration, is enzymatic or mechanical.

Growth of Mycelium and Host Reactions

Reaction type A. In a susceptible host, the mycelium is frequently intercellular, forming few or no haustoria for as much as the first month of its development. The cortex cells adjacent to the advancing mycelium become abnormally granular and stain more heavily than normal cells of the same kind. From microchemical tests it can be shown that these cells contain an abundance of tannin, while those more recently surrounded by mycelium are more nearly normal (Fig. 4, B). These latter cells, even at a later stage, do not become so densely granular as do those first attacked. It appears that the cells gradually lose their ability to produce or acquire tannins or else the necessity for tannin formation is withdrawn. It will be noted in figure 4, B, that the haustoria are few and decidedly stunted.

As the mycelium continues to spread through the cortex, the nutritional relationship seems to change, for many large haustoria are formed. The hypha becomes very much constricted as it passes through the minute opening that it produces in a cell wall to form a haustorium (Fig. 3, A). The host cells thus parasitized appear in no way to depart from their normal condition. We have here, then, an example of the almost mutualistic relationship of host and parasite so often reported in the literature, especially in the case of rusts (3, 24, 25, 37, 38, 40).

The early reaction to infection is not always like that just described. Sometimes, in the susceptible host the mycelium forms large haustoria

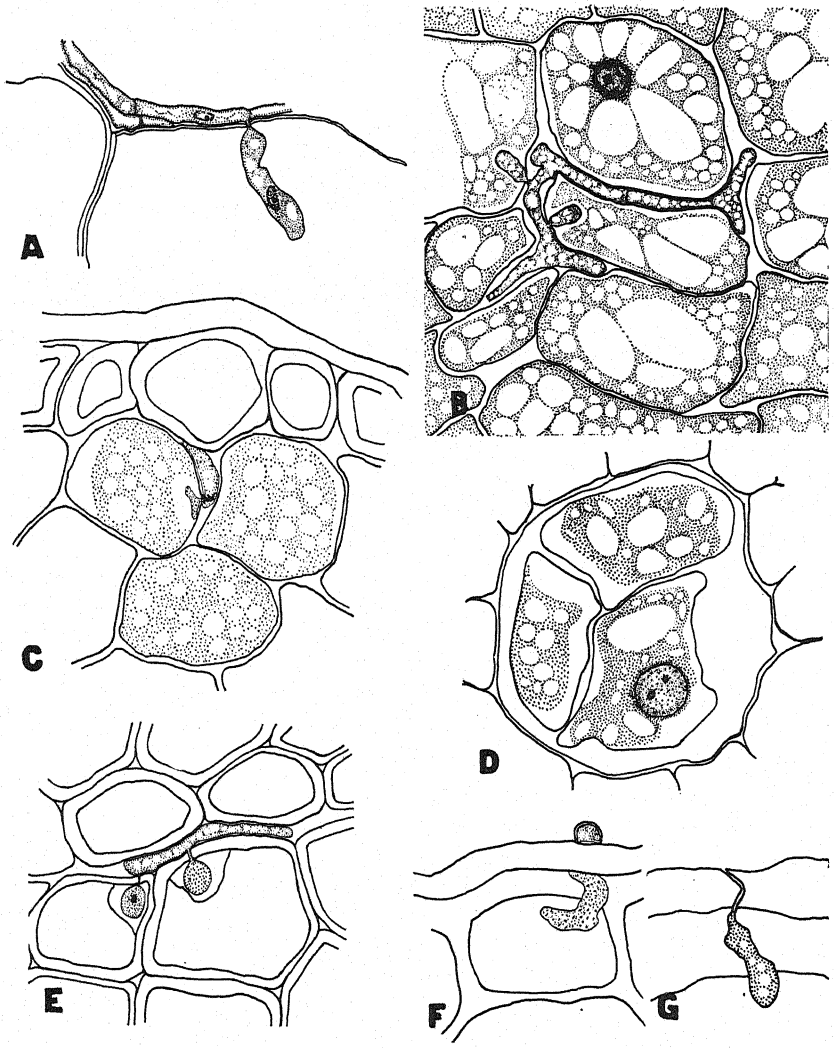
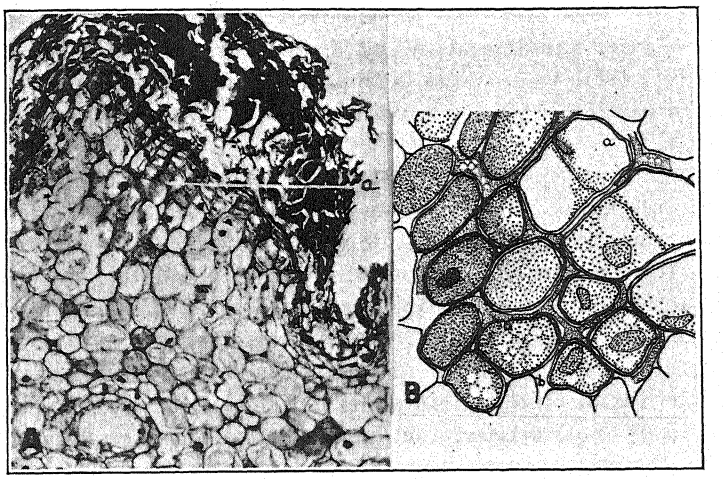


FIG. 3. A. Portion of cortical cell of susceptible host showing mature haustorium. ×1000. B. Portion of cortical region of susceptible host showing intercellular mycelium and penetration of haustoria. ×900. C. Group of epidermal and cortical cells of resistant host showing formation of small lobed haustorium. ×750. D. Giant cell from pathological xylem of resistant stem. ×700. E. Tracheid cells of susceptible host showing haustoria encased in lignified callosities. ×930. F. Epidermal cell of susceptible host showing germ tube which has penetrated the cuticle and is growing over the surface of the epidermal cell. ×950. G. Portion of wall of epidermal cell of resistant host showing the path of penetration of the germ tube through the cuticle. ×1900.



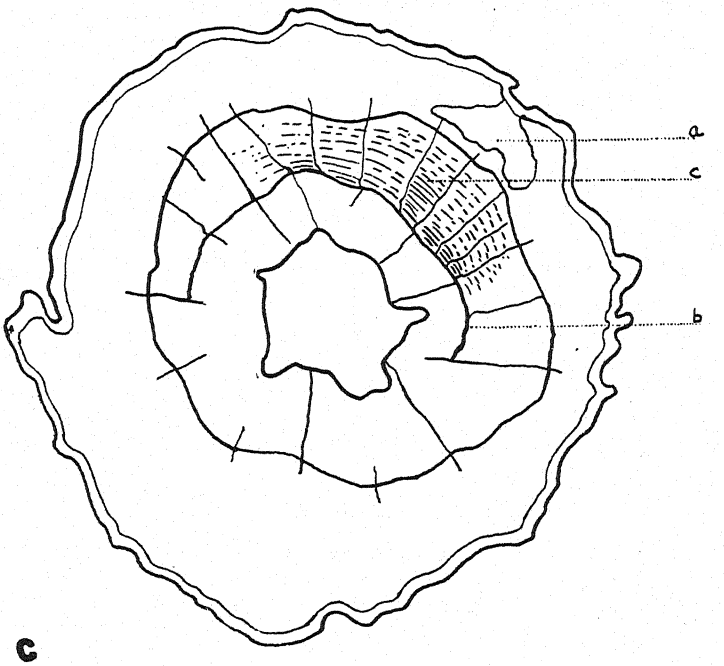


FIG. 4. A. Portion of transverse section of resistant stem of *Pinus sylvestris* one month after inoculation. a. Phellogen below necrotic area showing stimulation. B. Portion of cortical region of susceptible host directly beneath epidermis. The granular cells at the left are those first attacked by the fungus after penetration. These cells contain much tannin. Note stunted haustoria in cells a and b. ×300. C. Diagram of transverse section of resistant stem three months after inoculation. a. Necrotic area in cortex and phloem where cells have died following fungus invasion. b. Pseudo-annual ring formed by inhibited cambium derivatives. c. Region of tangentially elongated tracheids, bending of rays, and excessive formation of secondary rays. ×35.

directly after penetration of the epidermis. The cortical cells do not become filled with tannin and, in fact, appear perfectly normal (Fig. 3, B). In such cases there would seem to be an immediate adjustment of parasite and host without the preliminary hesitation and gradual adjustment as described above.

During the latter part of the growing season in which infection occurred, or the beginning of the following season, this semimutualistic balance becomes somewhat upset in that there is a decided stimulation of the host cells resulting in gall formation. Still, in the developing gall, little change can be noted in recently parasitized cells. A large number of the cells in the developing gall become gorged with starch and may be penetrated by haustoria. Starch is not confined to normal storage tissues, but may be found even in thin-walled tracheid cells. Some cells in the gall have undergone degeneration, and often cells adjacent to these contain much tannin.

It is notable that in reaction type A the mycelium may spread unhindered by any cork barrier. By the end of the season in which infection occurred, the mycelium has advanced to the cambium. Haustoria penetrate many of the newly formed nonlignified xylem elements. As these cells mature the haustoria become surrounded by heavy layers of cell-wall material (Fig. 3, E), forming structures similar to the lignitubers described by Fellows (17); these protuberances usually stain in the same manner as lignified membranes. The haustoria embedded within these protuberances appear to remain alive, but have not been observed to break through this heavy encasement. Much more delicate callosities are apparently formed about haustoria in invaded cortex, phloem, and ray cells.

Reaction Type B. In partially resistant plants the early stage of infection corresponds to that in susceptible plants in that tannin accumulates in cells adjacent to the mycelium. Within a few weeks after inoculation, it can be noted that a group of host cells has died. The cell walls are thickened, and the cells often contain brownish or yellowish globules. Mycelium can rarely be seen within or between these dead cells, but is abundant in adjoining areas, where it forms many haustoria. A suberized layer, usually 3 to 4 cells wide, is formed enclosing this necrotic area, as well as the infected tissue adjacent to it. The mycelium advances to this layer of wound cork and in many cases breaks through it, infecting the healthy tissue beyond. As a result, some of these infected cells die, a second cork layer may be formed, and the progress of the mycelium again checked. Three such alternating layers of necrotic tissue and cork have been observed in some twigs. When the mycelium finally gains a foothold and is no longer checked, a gall may be developed, but it is often

aborted or atypical. Usually, however, the mycelium seems to degenerate, becoming unable to break through the second or third cork barrier, and finally disintegrates. As the twig continues to grow, the bark is split and there is an exfoliation of the necrotic areas. This may continue for 2 or 3 seasons and may sometimes be accompanied by a generalized swelling in this area. This would appear to be due to the stimulation of the cambium in a manner probably similar to that described under reaction C.

Reaction type C. Sections through the discolored infection spots commonly found on all trees after artificial or natural inoculation, but frequently the only symptom of infection in the resistant trees, show small necrotic areas in which often no mycelium is evident. As soon after penetration as the fungus comes in contact with the subepidermal cells, these cells respond by becoming intensely granular with tannin, as described above. They also develop thickened cell walls, at least on the side near the mycelium. An occasional haustorium (Fig. 3, C) may penetrate these cells before this response occurs. As the mycelium makes feeble progress, the cortex cells first attacked continue to develop even thicker walls, and the cells, instead of remaining granular, become filled with large globules that, in unstained sections, appear yellowish brown. Microchemical tests would indicate that some of these globules are composed of gum, and others of oil or fat, both types being in some way associated with tannin. Haustoria in such collapsed cells occasionally retain nuclei and appear normal for a short time before their death and disintegration.

Sections examined one month after inoculation, at a time when these infection spots are visible macroscopically, show definite necrotic areas, often extending as far as the phellogen (Fig. 4, A). Many cells are collapsed, the majority contain the globules described above, the cell walls are thick and often lignified, and the entire area takes a very heavy stain with safranin. The cells adjacent to such areas show a stimulation in that the nucleus is increased in size and the cells show an increased staining ability. In the nearby phellogen regions, tangential division of the cells has occurred to a greater extent than in the normal portions of this tissue. A search for mycelium or haustoria among this group of dead cells has been uniformly unsuccessful. These necrotic areas, then, may be considered similar to those reported in various resistant hosts infected with rusts (3, 24, 25, 33, 40). These regions of necrotic tissue are soon sloughed off as the twig continues to develop.

In these resistant twigs, as mentioned above, an occasional swelling may form, as though galls were to be produced. In the development of such areas, it would appear that there is a delayed response in the first-attacked cortex cells. The formation of tannins, the thickening of cell walls, and

finally the development of globules and the collapse of the cells do not accompany the early infection. Consequently some of the hyphae may progress to other normal cells before they are checked by the death of the first-invaded tissue. In this way the mycelium is able to reach the cambium during the first growing season. The hyphae are by no means abundant, but tend to be fragmentary and can be seen only with difficulty. During the progress of the parasite, the host cells directly back of and adjacent to the advancing hyphae have died, and there is consequently formed a wedge-shape area of necrotic tissue extending from the epidermis to the cambium (Fig. 4, C). The majority of the cells in this area are dead. Globular contents of cells and excessively thickened cell walls are evident. Some cells that have not died possess very large nuclei and may contain much starch or oil. This wedge-shape area is separated from the normal cortex and phloem by a layer of wound cork. Adjacent to this the cells show the result of stimulation in that they stain much more heavily than similar normal cells, and are often gorged with starch. Early in the growing season following that in which infection occurred, a layer of sclerenchyma is formed adjacent to the necrotic area and directly external to the wound cork. Any mycelium that may be distinguishable at this time appears to be dead and disintegrating.

Cambial development during the latter part of the first season results in the formation of extremely pathological tissue. A portion of the cambium, often embracing half of its circumference around the twig, may be almost completely inhibited in development (Fig. 4, C), while the rest of the cambium is developing normally. The few divisions that may occur in these inhibited cells yield derivatives that fail to mature but appear to collapse and die very soon after formation. Later in the same growing season, however, this inhibited tissue may become greatly stimulated. Individual cambial cells increase in size, especially in a tangential direction, often becoming 4 to 6 times as long as normal cells. The tracheids, as well as phloem elements derived from such cambium cells, are also very much elongated tangentially. The stimulus that brings about this pathological condition often continues to act during the second season after inoculation, although it appears to be greatly weakened, for the cambium and its derivatives gradually become normal.

The xylem elements, formed just prior to and during the attack of the fungus on the cambium, some of which may be invaded by haustoria, do not complete their differentiation, but in many cases remain thin walled, retain cytoplasm and nuclei, and may be excessively pitted. Quantities of starch and oil may be stored in such juvenile xylem cells. Giant cells are frequently produced (Fig. 3, D) and it is not uncommon to find the

formation of tylosis-like cells extending through a pit in one tracheid into another. According to Chrysler (7) this phenomenon in pine is a result of wounding. Resin ducts in such areas are filled with tylosis-like cells in which starch may be stored. Rays developing through such abnormal areas are composed of abnormally large cells in which also much starch and oil may be stored. There is a very noticeable bending of these rays (Fig. 4, C), the change in direction of growth occurring at the point where the cambium is inhibited and its derivatives killed.

The study of sections through infected areas, cut during the latter part of the third year after inoculation, usually shows that the stem tissues have recovered from the effect of the stimulus. The juvenile tracheids and the layer of dead-inhibited-cambium derivatives in the first-year xylem compose what appears like a pseudo-annual ring extending half way or more around the stem (Fig. 4, C). External to this may be a considerable area of tangentially elongated or abnormally large tracheids. The tracheids of the late second-season and entire third-season growth have become gradually shorter tangentially, and those nearest the cambium are entirely normal. The bending of the rays at the position of the pseudo-annual ring is evident throughout the abnormal portion of the stem. An unusually large number of secondary rays arise as the tissues become more nearly normal (Fig. 4, C). The cambium as well as the more recently developed phloem tissues appear normal. The wedge-shape necrotic area has become completely surrounded by wound cork and sclerenchyma. The entire area stains heavily with safranin, and many of the thickened cell walls appear to be lignified. During the next growing season, this area is gradually crowded out nearer and nearer to the exterior of the twig. Portions of it are gradually sloughed off and finally the whole area is thus removed. The study of the progress of these later stages is often complicated by the fact that there may be a secondary infection, possibly by a wood-rotting fungus.

From the standpoint of pathological anatomy, the juvenile tracheids that retain cytoplasm and nuclei and are checked in their differentiation typify a hypoplasia (23). On the other hand, the formation of enlarged tracheids, giants cells, and tyloses in tracheids and resin ducts would represent a hyperplastic condition. The entire area of pathological xylem can be considered as wound wood.

In view of the large amount of pathological tissue formed in these stems, there might be some doubt about referring to them as resistant. However, since the fungus is definitely checked in these tissues, never comes to fruition, and furthermore does not sufficiently stimulate the tissues to produce a gall, these stems may well be considered resistant.

PHYSIOLOGICAL STUDIES

Chemical Analyses

Collections of twigs of the current season's growth were made from 12 resistant trees varying in age from 8 to 15 years. Each tree had been artificially inoculated for several successive years, but had failed to produce any galls, the reaction to infection being always of type C. These trees were typical of the entire group under consideration, as they were chosen from the different plots, some growing in partial shade and others in full sunlight, some growing on hillsides and others on level ground. Collections also were made from 12 susceptible trees, 8 to 15 years old and growing under conditions similar to those described above. Each tree had numerous mature galls. These collections from susceptible trees were further divided into one group consisting of twigs without galls and into another group containing only galls.

The selection of samples for analysis was made by dividing each twig into several portions and making a random choice of these parts after they had been thoroughly mixed.

Analyses have been made particularly for tannin,[6] potassium, and calcium.[7] The results expressed in per cent by weight are as follows:

	Resistant	Susceptible	Galls
Moisture-free basis			
Soluble solids	12.9	13.8	16.3
Non-tannins	8.7	9.9	11.2
Tannin	4.2	3.9	5.1
As plant ash			
CO_2 and C free ash	1.2	0.93	
CaO	29.86	33.32	
K_2O	41.40	18.09	

Perhaps the two points of significance in these results are the great excess of potassium in the resistant plants and the higher content in the galls of both tannin and other soluble solids. It is recognized that these results should not be given undue weight, as they represent but one determination. Yet the striking difference in potassium content of these two carefully chosen samples may be of considerable significance. The rôle of potassium in the plant (27) is thought to be closely connected with the synthesis of carbohydrates and probably of proteins and oils also. If,

[6] The tannin analysis was made by the Division of Industrial Farm Products, Bureau of Chemistry and Soils, United States Department of Agriculture, Washington, D. C.

[7] The potassium and calcium analysis was made by Mr. H. J. Hallowell, Consulting Chemist, Philadelphia, Pa.

then, resistance may have some nutritional basis, this potassium relationship may be fundamentally related to it. It has been repeatedly shown (13, 30, 31, 32), that plants grown with a lack of potassium are more susceptible to disease than those grown with sufficient potassium.

Analyses of samples of soil from both surface and subsoil[8] collected from the base of three resistant trees and three susceptible trees growing under the same conditions in very close proximity have failed to reveal any significant difference in potassium or calcium content. The following results are based on air-dried soil:

Trees	Sample	Potassium %	Calcium %
Resistant	Surface soil	1.24	0.60
	Subsoil	2.29	0.80
	Surface soil	2.20	0.60
	Subsoil	2.29	0.73
	Surface soil	2.29	0.52
	Subsoil	2.49	0.47
Susceptible	Surface soil	2.65	0.63
	Subsoil	2.53	0.94
	Surface soil	2.61	0.58
	Subsoil	2.69	0.74
	Surface soil	2.37	0.62
	Subsoil	2.52	0.68

Relation of Cell Sap to Growth of the Fungus

In a further attempt to note some physiological difference between susceptible and resistant trees, a study was made of the effect on the fungus of juices extracted as described above from noninfected susceptible and resistant trees. Since it has not been found possible to produce mycelium by culturing rusts on any sort of culture medium, the criterion of fungus growth could be only spore germination and a comparatively slight growth of the germ tube. The spores were sown in drops of the liquids on depression slides and kept at room temperature. In view of the fact that tannin has been found in some cases to be a deterrent to fungus growth, the effect of various strengths of tannin dissolved in distilled water has been similarly studied.

The results of these studies are summarized in table 1. It will be noted that the juices from susceptible and resistant trees have approximately the same relationship to the growth of the fungus. The resistant juice does not show the deterring effect that might be expected, but even would

[8] The soil analyses have been made by the Division of Soil Fertility, Bureau of Chemistry and Soils, United States Department of Agriculture, Washington, D. C.

appear to increase the percentage of germination. The results of the study with tannin indicate conclusions similar to those reached by Cook and Taubenhaus (10), *i.e.*, that low percentages of tannin solution may stimulate fungus growth, perhaps acting as food, but that higher percentages may inhibit or prevent growth.

Physico-chemical Constants of Cell Sap

Samples of juices freshly expressed from resistant and susceptible twigs as described above were used to determine osmotic pressure by means of the Beckman thermometer, and hydrogen-ion concentration by means of the potentiometer. The following constants have been secured:

Sample	pH	Osmotic pressure in atmospheres
Resistant	3.84	13.60
Susceptible	3.76	13.69

The rather close correspondence of these figures would indicate very little difference between the resistant and susceptible trees in these respects.

Injection Experiments

The formation of abnormal tissues in the resistant twigs, even after the mycelium had been checked and had disintegrated, would indicate that the stimulation may be due to some chemical formed by the disintegrating mycelium or host cells. Two sets of injection experiments have been performed to attempt to induce such abnormal changes by chemical means. An extract was made of mature galls by grinding them with sand and distilled water and filtering the liquid through a Seitz filter. A portion of the filtrate was shaken with aluminum hydroxide and refiltered, much of its brownish color being removed by this process. Another extract was prepared by germinating spores until the germ tubes had reached their maximum length. They were then frozen in distilled water so that the germ tubes were ruptured and the liquid was then filtered.

These three extracts were introduced into the plant tissues, both by hypodermic and capillary injection.

In the same manner 1 per cent and 0.1 per cent solutions of oxalic acid were injected into twigs. Controls of injections of distilled water and simple needle punctures were made on several trees.

During the course of 3 months some of the injected twigs developed slight swellings and resinosis. Collections were made at intervals of 1 month for 3 months, and the tissues prepared and stained as described

TABLE 1.—*Germination and growth of germ tube of spores of Peridermium sp. sown in various dilutions of tannin and in juices extracted from susceptible and resistant trees*

Medium	No. of spores examined	No. of spores germinated after 4 hours	Germination per cent	Average length of 10 longest germ tubes after 4 hours μ	Average length of 10 longest germ tubes after 18 hours μ
Juice extracted from uninfected resistant trees	1135	88	7.8	57	192
Juice extracted from uninfected susceptible trees	1143	32	2.8	68	192.5
Tannic acid— 1%	780	0	.....	.....	.....
" " 0.1%	316	0	.....	.....	.....
" " .075%	1060	185	17.4	105	.....
" " .05%	565	53	9.4	88.2	.....
" " .025%	1200	77	6.4	74.2	.....
" " .01%	900	40	4.4	30.0	225
Distilled water	360	0	.....	.....	.....
Tap water	800	20	2.5	83.8	210

above. A comparative study of the control twigs and those injected with chemicals has revealed no cell or tissue abnormality other than could be attributed to wounding. Adjacent to the wound caused by the needle or tube several cell layers had collapsed and died. Internal to this area a cicatrice layer had formed about the entire wound. For several cell layers internal to this there was a decided stimulation of cells in that they stained more heavily than similar normal cells and the nuclei were often larger than in normal cells. There appeared to be no stimulation of the cambium due to the diffusion of the extracts or acid into this tissue.

DISCUSSION

Studies of resistance among plants to parasitic organisms have revealed the fact that this resistance may be morphological, physiological, or functional in nature. It has further been shown that the resistance of a plant may depend, not on a single phase, but may perhaps be determined by all phases.

In the present study attempts have been made to correlate resistance with some morphological feature or with some physiological aspect of the

plant and, in a study of the reactions of the plants to inoculation, to note any differences that might help to explain resistance.

Twigs from noninfected resistant and susceptible trees have shown no histological or microchemical differences that might account for the different reactions to invasion by the rust. In relation to the physiological aspect, the only outstanding difference noted is the potassium content of the noninfected twigs. Perhaps too much significance should not be placed upon this fact. Yet, in view of the generally accepted rôle of potassium in the plant as a catalyst in carbohydrate formation, the relationship of the excess of potassium in resistant trees to their resistance might well be investigated further.

It is, however, from a study of the reactions of the plants to inoculation that most can be learned in this case about the nature of resistance. The fungus in one plant may progress unimpeded and establish itself in an almost mutualistic relationship with the host, while in the tissues of another plant, or in the tissues of another portion of the same plant, the host cells may be killed almost immediately and the fungus partially or completely checked in its progress. The cause of these markedly different host reactions is not easily determined.

It is well first to consider how the results of this study may be correlated with the starvation hypothesis suggested by Ward (37). The complete checking of the parasite by the collapse and death of invaded host tissue might be considered to be the result of the inability of the fungus to secure food from its living host cells, and its further inability to adapt itself to any saprophytic mode of life.

The occasional spread of the mycelium from such necrotic areas, especially when the host reaction has been a mild or gradual one, would appear to depend upon the vigor of the fungus. This vigor is without doubt determined by the amount of food the hyphae are able to secure before the necrosis sets in. This food may be transferred to the advancing hyphal tips, which are thus able to grow beyond the dead tissue areas. If the fungus in its food absorption can keep ahead of the host necrosis, it may be able to advance even to the cambium during the first season. If, however, the host necrosis becomes more rapid, the mycelium may lose its vigor and degenerate since it is surrounded by dead host tissue. In the susceptible host the cells would appear to be stimulated to acquire additional food material for the use of the fungus. The fungus thus increases in vigor and advances unhampered through the tissues.

True[9] has noted that even in the most susceptible Scotch pine trees very heavy inoculations with this same species of Peridermium may result in no

[9] True, R. P. Gall development on *Pinus sylvestris* attacked by the Woodgate Peridermium and morphology of the parasite. In manuscript.

gall formation, but in the cracking of bark characteristic of reaction type B. This fact might also be explained on a nutritional basis. The numerous mycelia spreading from several infection centers may defeat each other in their struggle to secure food from the host and none may be able to establish itself.

The same explanation may be given for the failure of many infection centers to develop in the susceptible host, although from one such center the mycelium spreads and produces a gall. The mycelium that is able to establish an immediate congenial relationship with the host cells would appear to secure almost a monopoly on the food supply.

As Wellensiek (40) has pointed out, starvation may result not only from the entire lack of individual food substances, but may be brought about by only a slight variation in the quantity of some food material. The apparent difference in potassium content between suceptible and resistant twigs may indicate some carbohydrate excess or deficiency, as suggested by Mains (24).

The failure to demonstrate by injection experiments the presence of any toxic material in the fungus should not invalidate an assumption that toxin production may take place in the living cell invaded by haustoria. Allen (1) has considered that such an assumption may best account for resistance in wheat. There is evidence that the death of host cells precedes the death of the invading haustoria; similar evidence has been obtained by Allen (1) and Stakman (33). It would thus appear possible that a toxic substance released by the fungus is responsible for the death of the host cells. The disintegrating cell contents in turn may release materials harmful to the haustorium. It also is conceivable that in these poisoned host cells the food available for the fungus may be rendered unavailable, and the haustorium will soon die from starvation. On the other hand, in reaction type A the toxin produces no visible injury, but stimulates the cells to increase in size and number. It would thus appear that cells showing the resistant reaction are hypersensitive to the excretion of the fungus.

It has been noted that a layer of cork cells develops about the infected tissue in both reaction types B and C. This is later followed by the formation of a continuous layer of sclerenchyma cells about this region. This corky layer might at first be considered to have some definite relation to resistance. However, the same type of tissue may be formed as a reaction to mechanical wounding. Invaded tissues that respond by collapsing and becoming necrotic are in reality wounded tissues and function as a pseudo-cicatrice layer (42). The continuous layer of cork may then be considered a cicatrice layer and thus to represent solely a response to wounding. The usual lack of cork development in those infected areas in the susceptible host from which galls develop is apparently due to the fact that here the

host cells are not injured or wounded. Only when the fungus is low in vigor and apparently unable to secure sufficient nourishment is it checked by this cork layer. Thus this layer should be considered only meagerly related to resistance.

The accumulation of tannin in those cortical cells first attacked by the fungus has been found accompanied by at least a partial checking of the mycelium. While tannin also has been observed in abundance in tissues of wounded control twigs, it would seem to have some bearing upon resistance in this case. As has been pointed out, the mycelium may spread rapidly in the susceptible host without any accumulation of tannin in the host cells. In other susceptible twigs the mycelium makes slow progress until it grows beyond the tannin-containing tissues. In some resistant, as well as some susceptible twigs, the mycelium makes no progress beyond the tissues in which tannin accumulates. It is quite probable that other factors besides tannin accumulation are involved in this partial or complete retarding of the fungus. It is not clear in just what manner tannin may help to bring about this reaction. Haustoria growing into such tannin-filled cells are not usually killed until the cell itself has died. The study of spore germination and growth of germ-tubes in various percentages of tannin might indicate that the action upon the parasite is somewhat toxic.

This accumulation of tannin in host cells might well be considered as representing the "local immunity" suggested by Dufrénoy (14) for similar accumulations of phenolic compounds.

SUMMARY

A comparative study of stem tissues and extractives of *Pinus sylvestris* trees susceptible and resistant to a species of Peridermium has been made in order to determine the nature of resistance to this parasitic fungus.

No correlation has been found between any morphological feature of the noninfected host and the resistance to infection.

Three general types of reaction to inoculation may be distinguished: (1) typical gall formation on the susceptible host; (2) cracking of bark and slight resinosis sometimes followed by the formation of atypical galls on the partially resistant host; (3) formation of small necrotic areas on twigs followed sometimes by swellings that do not increase in size after the first or second year but are gradually sloughed off.

In the most susceptible plants the mycelium may actively grow in the host and live in something of a mutualistic relationship. This results in stimulation of living cells and the formation of a gall.

In the most resistant trees the host cells are killed immediately after invasion. The fungus also soon dies and the necrotic area is sloughed off.

Often, in both resistant and susceptible plants, the first-invaded cells

may become filled with tannin. This reaction may represent a local immunity.

In those resistant trees that develop swellings, mycelium grows to the cambium and then dies. Areas of necrotic tissue extend from epidermis to cambium. The cambium becomes stimulated to form quantities of wound wood including tangentially enlarged tracheids, giant cells, and tyloses in tracheids and resin ducts.

In the resistant reaction, the necrotic area becomes surrounded by cork and later by sclerenchyma. This probably represents a normal reaction to the wounding of cells, and may play a small part in resistance.

No correlation has been observed between the osmotic pressure or hydrogen-ion concentration of cell sap and resistance.

A greater amount of potassium has been found in noninfected resistant stems than in susceptible stems. This is thought to have some possible relationship to resistance on the basis of nutrition.

It is considered that the liberation of toxin into cells by the haustoria may cause the death of the hypersensitive cells of the resistant host. The fungus is thus deprived of its food supply and soon dies. In the susceptible host, however, toxin does not kill the cells at once but simply stimulates them to gall formation.

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GRAFT VERSUS INSECT TRANSMISSIONS OF CURLY TOP IN TOMATOES (TOMATO YELLOWS)

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PROCEDURE

The curly-top virus, causing a disease in tomatoes known as tomato yellows (4), was previously transmitted by the writer through approach grafts from diseased to healthy tomato plants (6). The technique employed in these early experiments also was used in subsequent trials. In addition to very young seedlings, older plants were used both in the greenhouse and in the field. Slices about 1 inch long and a depth of $\frac{1}{4}$ to $\frac{1}{3}$ of the diameter of the stem were cut off from the opposite sides of each plant, the exposed surfaces were brought in contact and the two plants were tied firmly together. Because of its flexibility, the electrician's rubber splicing compound was found very well suited for the purpose and caused no injury to the plants. The approach graft, when completed, appeared as shown in figure 1. Only in certain exceptional cases did growing plants grafted in this manner fail to unite.

One of the plants of the graft pair was exposed to viruliferous beet leaf hoppers, *Eutettix tenellus* Baker, either before or after grafting, as required by the experiment, and the effect of this exposure on both plants was studied. As a rule, 10 insects were used to each plant, confined in a celluloid cell tied over a leaf or a tip of the plant (5). The insects were removed after about 5 days of feeding, and the tape was cut away about a week or 10 days after grafting. If at that time a union was formed the cion of the insect-inoculated plant was severed from the stock just below the graft region.

EXPERIMENTS

The original purpose of grafting work in connection with tomato yellows was to find a simple and efficient method of transmitting this disease to take the place of the customary slow and cumbersome procedure of the beet leafhopper transmission. Had grafting proved to be such a method, it would have been expected that the diseased tomato cions would provide the inoculum. Accordingly, the earlier trials dealt with grafting small diseased shoots or buds on healthy tomato plants. These experiments resulted in almost complete failure, as only an occasional bud would form the union and

[1] Acknowledgments are due to Mr. E. C. Raby of the Citrus Experiment Station, Riverside, Calif., for valuable assistance during the early greenhouse stages of this work and to Mr. Paul Evans, of the Bureau of Plant Industry, U. S. Dept. of Agr., whose help was indispensable during the later field trials.

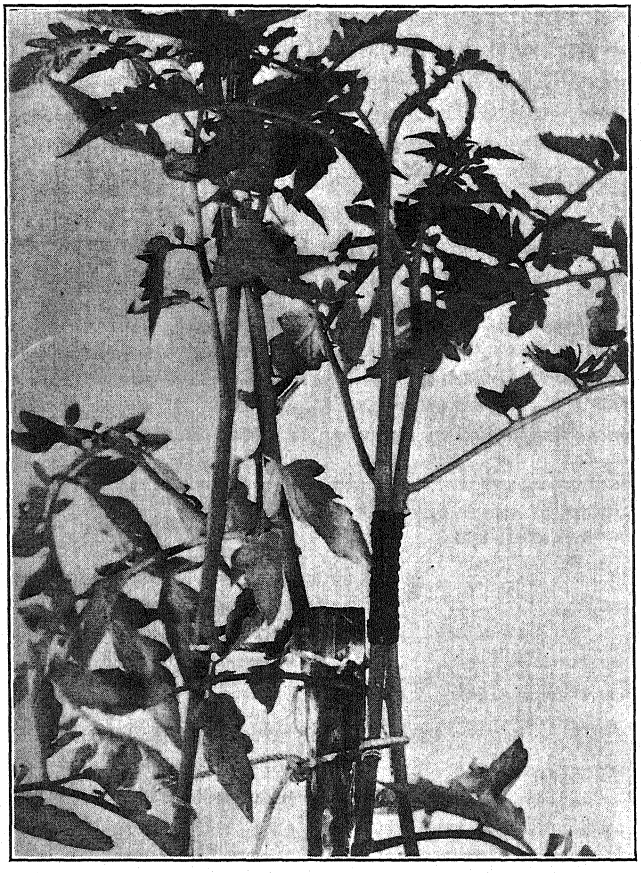


FIG. 1. A pair of tomato plants united by means of an approach graft for the purpose of transmitting curly-top virus from an insect-inoculated plant to a healthy plant.

only one out of 21 plants so grafted contracted the disease. The diseased cions and buds had very little if any vitality or power for regeneration. No further attempts were made to graft diseased cions on healthy plants.

Since, however, it was observed that often plants show a tendency to regenerate (2), it was of interest to ascertain whether whole diseased plants may not possess greater ability to form successful graft unions with healthy plants than small branches of these plants. This led to the subsequent experiments with approach grafting. Such a method of inoculation could not, however, be looked upon as a proper substitute for the usual procedure of insect transmission, as it required the latter to begin with and then the graft in addition, but it gave other interesting data for the study of the virus passage between a pair of graft-united plants.

The approach grafts at the very outset proved to be a much better means of obtaining a perfect union of the grafted plants as well as of transmitting the disease to healthy uninoculated companions of the graft pair. However, when plants showing fully developed curly-top symptoms were used for grafting, the percentage of failure to form a union with healthy plants was still too high and the transmission practically nil. Table 1 shows that in the group of 13 grafted pairs, of which one plant showed well-developed symptoms of yellows, only 1 healthy plant contracted the disease out of 8 successful grafts.

TABLE 1. *Results of grafting diseased and healthy plants. Stages of tomato yellows: S—slight, leaves roll or purple, but no yellowing; M—medium, leaves begin to turn yellow and show more rolling and vein purpling; Y—severe stage, very little of green color left. PU—perfect union; No U—no union. Grafts were made in a greenhouse at Berkeley, California, on December 22, 1930. Results recorded on January 6, 1931*

Conditions of diseased plants at grafting	Final results
S	PU, no transmission, diseased plant dead
S	PU, no transmission, diseased plant recovered
M	PU, disease transmitted, diseased plant dying
Y	PU, no transmission, diseased plant dead
Y	"
Y	"
Y	"
Y	"
Y	No U, no transmission, diseased plant dead
Y	"
Y	"
Y	"
Y	"

It was decided, therefore, not to wait for the development of symptoms, but to graft the plant exposed to viruliferous insects with a healthy noninoculated plant, either simultaneously with the exposure to insects or shortly afterwards. For the sake of brevity, the individuals on which the insects were fed are called inoculated plants, whereas those grafted to these inoculated plants are called grafted plants. In all counts given in the following tables the pairs that formed no union are excluded.

As shown by a series of 15 tests, with the number of good unions varying in individual tests from 12 to 98, a greater number of plants invariably became diseased on the inoculated side than on the grafted side. Apparently, in some cases, the virus present in the inoculated plant found some unsurmountable obstacle in crossing the graft bridge connecting the diseased and

the healthy plants. The exact differences in these 15 tests are given in table 2.

TABLE 2.—*Showing curly-top transmission through approach grafts in tomatoes*

Group	Total No. of pairs grafted	No. diseased plants in		Differences between (a) and (b)
		(a) Inoc. group	(b) Corresponding grafted group	
I	19	11	8	3
II	12	9	7	2
III	15	10	9	1
IV	23	21	11	10
V	24	24	7	17
VI	22	21	5	16
VII	24	23	11	12
IX	48	46	11	35
X	44	40	37	3
XI	28	20	16	4
XII	28	22	6	16
32/2	95	87	77	10
32/3	98	94	78	16
33/A	15	8	7	1
33/B	15	3	2	1
Total	510	439	292	147

It should be noted that, so far, in no case did the disease appear in any plant of the (b) group without showing in the corresponding plant of the (a) group.

That the unfailingly smaller number of affected plants in the group of individuals grafted with insect-inoculated plants, as compared with the latter, is significant may be seen more clearly from table 3 where actual figures are changed to percentages of the total number of inoculated pairs forming perfect unions. In the preparation of tables 3 and 5 to 8 inclusive, Love's modification of Student's method of statistical evaluation (3) has been followed for its simplicity and adaptability to small numbers in paired experiments. The Z values were calculated from the formula:

$$m \div \sqrt{\frac{\sum (d-m)^2}{n}}$$

in which m is the mean of differences between a and b (with d representing individual differences between a and b) and n, which denotes the number of trials or samples. In accordance with the Z and n values, odds were taken from Love's table. Groups a and b in table 3, 7, and 8, while not independent variables, form natural pairs with reference to the virus movement from one plant to the other and from cion to stock.

TABLE 3.—*Indication of relative significance of slack in the curly-top virus transmission in tomatoes through the graft*

Group symbol	Total No. of pairs grafted	Percentage infected of the total grafted		Differences		
		In the insect inoc. (a)	In the healthy grafted (b)	a–b (d)	d–m	$(d-m)^2$
		Per cent	*Per cent*			
I	19	57.1	42.0	15.1	−15.6	243.36
II	12	72.2	61.1	11.1	−19.6	384.16
III	15	66.0	58.9	7.1	−23.6	556.96
IV	23	92.6	44.9	47.7	17.0	289.00
V	25	100.0	31.3	68.7	38.0	1440.00
VI	22	95.0	21.7	73.3	42.6	1814.76
VII	24	95.9	45.8	50.1	18.4	338.56
IX	48	95.9	22.9	73.0	42.3	1789.29
X	44	87.7	82.5	5.2	−25.5	650.25
XI	28	70.2	55.8	14.4	−16.3	265.69
XII	28	77.3	22.7	54.6	23.9	571.21
32/2	95	91.6	81.0	10.6	−20.1	404.01
32/3	98	95.9	79.6	16.3	−14.4	207.36
33/A	15	53.3	46.6	6.7	−24.0	576.00
33/B	15	20.0	13.2	6.8	−23.9	571.21
				460.7		10101.82

With the data presented in the above table $Z=\frac{30.7}{25.9}=1.2$, and odds approximately 3332 : 1 against the occurrence of differences as great as those obtained in this experiment being due to chance alone. This being the case, it was logical to assume that if several plants (instead of two) were grafted together in chain-like series, the plants farthest removed from the insect-inoculated plant would show a smaller percentage of the disease than the plant directly grafted to the inoculated one. To ascertain such a possibility, a field experiment was conducted in which 4 plants in each hill were grafted as illustrated by the diagram in figure 2. The results of this experiment (Table 4) show a tendency toward a gradual decrease both in severity and in percentage of the disease. The severity in this case was judged by the number of dead plants.

It is apparent, even from a superficial examination of tables 1 and 2, that there is a considerable variation between differences obtained in the amount of the disease on two sides of the graft union. A closer analysis of the circumstances under which these grafts were made indicated that a certain correlation existed between the time elapsed from the moment of inoculation to the moment of graft and the degree of success of the graft

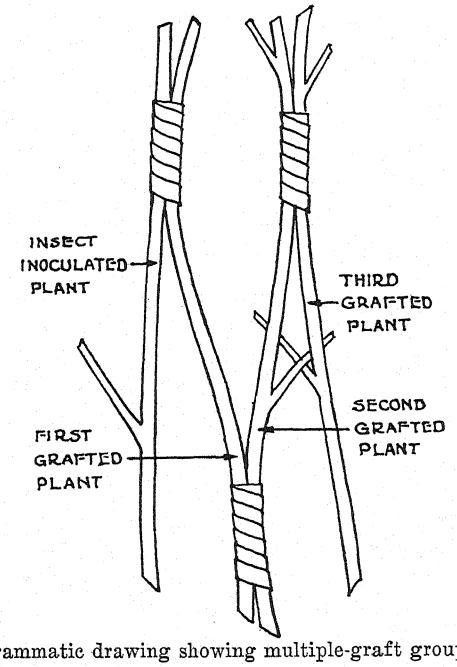


FIG. 2. A diagrammatic drawing showing multiple-graft group used in the curly-top transmission studies.

transmission of the disease. In accordance with this observation all grafts were grouped into 3 classes as follows: (I) advanced grafting group, comprising those pairs that were grafted several (usually 5 or 6) days prior to the inoculation of one member of the pair; (II) simultaneous grafting group, in which inoculation of one member immediately followed the grafting operation; and (III) delayed grafting group, comprising those pairs of which one member was inoculated several (usually 5 or 6) days prior to its

TABLE 4.—*The development of curly top in tomatoes with quadruple grafts*

Number of affected and dead plants	Inoc. plant	1st grafted plant	2nd grafted plant	3rd grafted plant
Row 1 (15 hills inoc.):				
Total No. of affected plants	14	13	11	10
Total No. of dead plants	11	8	6	6
Row 2 (16 hills inoc.):				
Total No. of affected plants	16	10	10	9
Total No. of dead plants	15	5	3	2

being grafted to a healthy plant. Correspondingly, tables 5 and 6 give the relative results of these experiments and show that the best transmission of the curly-top virus through the approach-graft union was obtained when the inoculation of one member of the pair was made almost simultaneously with its grafting to the other healthy member. No advantage was secured from an advanced grafting; there may have been even a slight disadvantage. Significant differences developed with a delayed grafting, giving, in this case, the poorest disease transmission.

TABLE 5.—*Simultaneous vs. advanced grafting of diseased with healthy tomato plants (percentage of infection)*

Group symbols	Grafted simultaneously with inoc. (a)	Grafted 5–6 days in advance of inoc. (b)	Differences		
			a–b (d)	d–m	(d–m)²
1932 1	94.4	84.4	10.0	5.4	29.16
2	80.9	75.0	5.9	1.3	1.69
3	73.7	85.0	−11.3	−15.9	252.81
4	70.0	76.2	− 6.2	−10.8	116.64
5	80.0	84.4	− 4.4	− 9.0	81.00
1933–	46.6	13.2	33.4	28.7	823.69
			27.4		1304.99

Accordingly, $Z = \frac{4.6}{14.75} = .31$ and odds = 2.85 : 1 against the above differences being due to chance, as they probably are.

TABLE 6.—*Immediate vs. delayed grafting of diseased with healthy tomato plants (percentage of infection) in development of curly top*

Group symbols	Time of grafting after the removal of insects		Differences		
	24 hrs. or less (a)	2 days or more (b)	a–b (d)	d–m	(d–m)²
	Per cent	*Per cent*			
I. III	90.0	72.7	17.3	− 8.9	79.21
IV, V	66.0	29.2	36.8	10.6	112.36
VI, VII	47.8	23.8	24.0	− 2.2	4.84
IXa, IXb	45.5	4.3	41.2	15.0	225.00
Xa, Xb	87.7	77.3	10.4	−15.8	249.64
XIa, XIb	60.0	33.3	26.7	0.5	0.25
XIIa, XIIb	36.4	9.1	27.3	1.1	1.21
			183.7		672.51

In this case $Z = \frac{26.2}{9.8} = 2.67$ and odds = 2499 : 1 in favor of the above differences being significant.

As stated in the introductory chapter of this article, the tape uniting 2 grafted plants was removed 7–10 days after grafting and, if a good union was evident, the grafted inoculated plant was cut below the graft junction and the insect-inoculated cion was left to grow on the originally noninoculated plant. The final results as to the transmission of the disease were based on the symptoms developing in the cion and the plant to which it was attached. The appearance of the disease symptoms in the cion, however, was not always accompanied by their development in the parent stock from which it was severed, and *vice versa*. In this connection it was deemed of interest to ascertain whether the cion or the stock was resistant to the successful development of the virus. The data presented in table 7 show that, although there is a slight preponderance in favor of cions, the difference does not seem to be unquestionably significant.

TABLE 7.—*Percentage of curly-top infection in cions vs. severed stocks (bases) of tomato plants*

Group	Cions (a)	Bases (b)	a–b (d)	d–m	$(d-m)^2$
	Per cent	*Per cent*			
A–1 (19)	89.5	84.4	5.1	– 1.4	1.96
2 (16)	93.7	62.5	31.2	24.7	610.09
3 (20)	90.0	95.0	– 5.0	–11.5	132.25
4 (21)	95.2	80.9	14.3	7.8	60.84
5 (19)	89.5	89.5	0.0	– 6.5	42.25
B–1 (18)	94.4	100.0	– 5.6	–12.1	146.21
2 (21)	95.2	85.7	9.5	3.0	9.00
3 (19)	100.0	89.5	10.5	4.0	16.00
4 (20)	95.0	80.0	15.0	8.5	72.25
5 (20)	95.0	100.0	– 5.0	–11.5	132.25
1933 (30)	36.6	40.0	– 3.4	– 9.9	98.11
			66.6		1321.11

With $Z = \frac{6.5}{10.95} = .59$, odds approximately $= 22:1$ indicating that the differences obtained in this experiment might well be solely due to chance.

There was, on the other hand, a decidedly significant difference in the number of recoveries from the disease, the bases or stocks of inoculated plants showing this tendency to a much greater degree than the cions, as may be seen from table 8.

This gives $Z = \frac{13.0}{9.3} = 1.4$ and odds $= 908:1$, decidedly against the occurrence of the above differences as being due to chance alone.

TABLE 8.—*Percentage of recoveries in severed bases vs. cions of tomato plants*

Group	Bases (a)	Cions (b)	a–b (d)	d–m	$(d-m)^2$
A–1	5.3	5.3	0.0	–13.0	169.00
2	12.5	0.0	12.5	– 0.5	0.25
3	15.0	0.0	15.0	2.0	4.00
4	14.3	0.0	14.3	1.3	1.69
5	36.6	10.5	26.1	13.1	171.61
B–1	0.0	0.0	0.0	–13.0	169.00
2	4.5	4.8	– 0.3	–13.3	176.89
3	22.2	0.0	22.0	9.0	81.00
4	25.0	5.0	20.0	7.0	49.00
5	20.0	0.0	20.0	7.0	49.00
			129.0		871.44

DISCUSSION AND CONCLUSIONS

It is obvious that grafting in the curly-top work with tomatoes can not take the place of the standard insect-inoculation method if simplicity and promptness are desired. For the best graft transmission it is necessary for the diseased plants to be in a very early stage of the disease, preferably before the appearance of distinct symptoms, which means a preliminary insect inoculation. Even with these requirements fully satisfied a certain variable number of plants fail to obtain the virus from the infected cions with which they form a union. The reason for this failure is not definitely known although a suggestion may be seen in the character of the cell structure in the graft region. As shown by Dufrénoy and Shapovalov (1), even successful graft unions contain a barrier of degenerated cells between the living tissues of the two plants, which are only partially connected by bridges of hyperplastic cells. Therefore, it may be surmised that the passage of virus from one plant to another under such conditions is made difficult and sometimes, perhaps, impossible.

It is more difficult to understand the failure of the curly-top symptoms to appear in the stock when the inoculated cion served after its union with a healthy plant develops these symptoms and *vice versa.* If the size of the plant is to be considered a factor, then both, apparently, should have had even chances for overcoming the spread of the infectious principle, the cion as being a part of another healthy plant and the stock as comprising nearly all of the original inoculated yet vigorous individual (Table 7). This does not, however, explain why the stocks showed a greater ability to recover from the disease than cions after an early appearance of the symptoms (Table 8).

The following conclusions may be drawn from the experiments described in this paper:

1. The infection of one of a pair of grafted tomato plants with curly-top virus does not assure the passage of this virus to the second grafted plant.

2. This failure of the second grafted plant to contract the disease from the other (infected) plant through the graft union occurs even when grafting and inoculations are made simultaneously, or when grafting precedes the inoculation and it is much greater when grafting is delayed.

3. The insect-inoculated shoot (or cion) may fail not only to transmit the disease to the healthy plant with which it is grafted, but may recover entirely from all symptoms of the disease after being severed from its mother plant (or base) below the graft union, or may never develop the symptoms, regardless of the condition of the mother plant from which it was severed.

4. The base, when severed from a diseased cion as above, may likewise fail to show any curly-top symptoms or it may recover from the disease, although the cion may continue to develop these symptoms transmitting the infection to the healthy plant with which it is grafted.

5. The number of recoveries among the bases of the inoculated plants was decidedly greater than among the cions severed from them.

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THE INFLUENCE OF PHYTOMONAS TUMEFACIENS AND PHYTOMONAS RHIZOGENES ON THE ACTUAL ACIDITY OF CERTAIN LIQUID AND AGAR SUBSTRATA[1]

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INTRODUCTION

The physiological interrelations of the host plant and the bacteria of crown gall and hairy root present many complex problems requiring separate detailed study. One of these problems, the change induced by the growth of the organisms in the pH value of the bacterial colony and of the substratum, has formed the basis of the present studies.

Previous work on this subject has been confined to changes in the pH value in liquid cultures after a lapse of a set period of time (7), (1), (8), (12), (6), (9), (4). As far as could be ascertained, little if any work has been done either on the sequence of changes induced by the growth of these organisms, or on the changes induced on agar media. Indications that differences might exist between the pH value of an agar medium and that of the bacterial colony growing upon it were afforded by Hendrickson *et al.* (4). These authors found that if aniline blue were added to a yeast-infusion manitol mineral-salts agar medium, adjusted to the neutral point of the indicator, the colonies of the crown-gall organism assumed a blue coloration, while the substratum remained colorless. This raised the question whether the phenomenon was brought about by differences in pH value, absorption, or some other factor.

The incubation temperature appeared worth consideration because of the influence of temperature on the pathogenicity of the crown-gall organism. Riker (7) found that, whereas both host and parasite grow well above and below a critical maximum temperature range of 28°–30° C., galls were induced by inoculations on tomato kept below but not above this temperature range. For this reason it was considered advisable to make a study of the pH relations of *Phytomonas tumefaciens* at 26° and 32° C. Parallel with this experiment a similar one was carried on with a nonpathogenic culture of the crown-gall organism (4). It appeared likely that such studies would

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This work was done whilst holding a research scholarship from the British Ministry of Agriculture and Fisheries.

indicate whether or not pathogenicity was correlated with the pH-temperature relations.

MATERIALS AND METHODS

Cultures of the crown-gall organism designated A-6 and A-6-6 were employed throughout the investigation. A-6 is the progeny of a single-cell isolation made by Wright *et al.* (12) and is pathogenic. A-6-6 is the progeny of an isolation made from A-6 by Hendrickson *et al.* (4) and is nonpathogenic. The culture of the hairy-root organism used was designated C-10 and is the progeny of a single-cell isolation also made by Wright *et al.* (12). The cultures were tested for pathogenicity both before and after the experiment. In both tests A-6 and C-10 proved pathogenic, while A-6-6 proved nonpathogenic. On the completion of each series of experiments, the viability of the bacteria was demonstrated by the growth of subcultures taken from both liquid and agar cultures.

Carrot-infusion and yeast-infusion glucose mineral-salts media were employed throughout the experiment. The carrot-infusion medium was prepared by steaming 200 g. of minced carrot in one liter of distilled water for 1 hour. The pH value of this medium was not adjusted. The composition of the yeast-infusion glucose mineral-salts medium[3] was as follows: 1 per cent yeast-infusion, 100 cm.^{3}; calcium chloride, ($CaCl_2$), 0.1 g.; sodium chloride, (NaCl), 0.2 g.; magnesium sulphate, ($MgSO_4 \cdot 7H_2O$), 0.2 g.; dipotassium phosphate, ($K_2HPO_4 \cdot 3H_2O$), 0.2 g.; glucose, 5 g., and distilled water, 900 cm^3. In later experiments the amount of the dipotassium phosphate was increased to 0.5 g., thus obviating difficulties experienced from the drop in pH value during autoclaving. The pH value of the medium was usually in the region of 6.8 and no adjustment was made. Agar media were prepared by adding 2 per cent shredded agar to the liquid media.

The cultures employed for transfers to the experimental series were made on yeast-infusion slants. These were kept at room temperature (21° to 22° C.) and were used when 4 days old. Aqueous suspensions of the organisms were prepared by adding 10 cm^3. of sterile water to each of the slants.

The tube cultures used in the experiments contained 10 cm^3. of liquid medium, the plates approximately 25 cm^3. of agar medium. In sowing the liquid tube cultures, 2 drops of the aqueous suspension were placed in each tube by means of a sterile pipette. The agar plate cultures were sown with 2 streaks approximately 5 cm. apart, made by lightly passing a sterile cotton-wool swab, moistened with the aqueous suspension of the organism, over the surface of the medium. Cultures were incubated at 26° and 32° C. and in order to prevent evaporation the plate cultures were placed in round metal containers, each holding 30 plates. Occasional fluctuations in temperature occurred, which, however, never exceeded 1° C.

[3] Hereinafter referred to as yeast-infusion medium.

The pH values were determined in preliminary experiments with the aid of glass electrodes. The quinhydrone equipment also was employed, as described later. The circuit utilised in conjunction with the glass electrode was of the simple potentiometric type, with the inclusion of a vacuum tube, as described by De Eds (3). The glass electrode was of the membrane type, having an internal diameter of approximately 4 mm. This electrode was prepared after the manner described by MacInnes and Dole (5) and was used in combination with a saturated calomel half-cell. Calibration was carried out each time, both before and after use, against a series of standard buffer solutions. The method employed in taking the readings was as follows: the electrode assembly, comprising the electrode and a potassium chloride-agar bridge, was fastened to the horizontal arm of a dissecting microscope, a delicate control thus being maintained by means of the rack screw. The electrode assembly was then lowered either into the liquid medium or on the surface of the bacterial growth. With the agar cultures, 2 readings were taken on each of the 2 streaks, the end of the electrode assembly being washed with distilled water after each reading. Difficulties experienced from induced currents set up by the movements of the operator, were lessened by the use of a grounded copper-gauze screen surrounding the electrode.

An attempt was made to run a parallel series of determinations of the oxidation-reduction intensities of the cultures. The taking of readings on the liquid cultures proved simple, but all attempts to obtain reproducible readings on the bacterial growth on agar media failed. In consequence, further reference to this aspect of the problem has been omitted.

Quinhydrone electrode readings were taken on the same cultures in parallel with the glass electrode readings. The method employed in taking the readings on the bacterial growth on the plate cultures was as follows: the bacterial growth was removed from the surface of the medium with cotton-wool swabs and placed in small glass tubes, where it was diluted with approximately 3 cm^3. of carbon dioxide-free distilled water. Even greater dilution was found to have no effect on the value of the readings.

Often considerable variation was found in the glass electrode readings on different parts of the streaks, owing presumably to an unequal rate of growth, but by taking the pH value of the whole bacterial growth, with the quinhydrone electrode, an average was obtained that was considered preferable.

A comparison of 56 readings taken on a series of cultures with both the glass and quinhydrone electrodes showed that, on an average, readings taken by means of the quinhydrone electrode on the growth on agar media were 0.32 of a pH unit lower than those obtained by means of the glass electrode. Readings taken with both electrodes on liquid media were almost identical.

A comparison of curves drawn from data secured by means of both electrodes showed that they were closely related in form. Since these comparisons demonstrated that the quinhydrone electrode could be employed for the work on agar cultures without loss of accuracy, it was used exclusively in later studies.

In continuation of the work of Hendrickson (4) with the indicator medium, readings were taken on the agar medium both below and midway between the streaks of growth on the plate cultures, in order to determine whether any considerable difference existed between the pH value of the bacterial colony and that of the substratum upon which it was growing. The samples of agar were removed by means of a cork borer and were ground in a small glass tube into a paste with a suitable amount of quinhydrone, to which was added approximately 3 cm^3. of carbon dioxide-free distilled water. As with the bacterial growth, even greater dilution was found to have no effect on the value of the readings. With the liquid cultures, a sample was decanted and mixed with quinhydrone in the usual manner. All samples were agitated until a constant reading was obtained.

RESULTS

Four series of determinations were carried out, involving a total of approximately 1,400 cultures and 4,000 readings, including duplication on each cultures.

No greater differences were noted between results at the two temperatures than those that might be ascribed to the slower growth of the organisms at the higher temperature. For this reason, in order to economise space, the graphs drawn from data obtained at 32° C. have been omitted. The graphs of data obtained from one representative series at 26° C. are given in figure 1.

The pathogenic (A-6) and nonpathogenic (A-6-6) cultures of the crown-gall organism, growing on yeast-infusion liquid medium, although not parallel in action, did not lower the pH value of the medium beyond what might be expected from absorption of carbon dioxide, nor did they show much drop in the pH value of the bacterial mass on the agar medium. These observations are in accord with the report by Riker *et al.* (8) that this organism does not produce acid from glucose.

A slightly greater fall in pH value was brought about by the growth, on agar medium, of the nonpathogenic strain of the crown-gall organism (A-6-6), than by the pathogenic strain (A-6).

The sharp initial fall in the pH values of the agar control in the series presented in figure 1 was not of constant occurrence. In other series the agar controls varied from having no initial drop to this extreme.

In cultures of the hairy-root organism (C-10), which produces acid from glucose (8), it was seen that, whereas in the liquid medium there was a

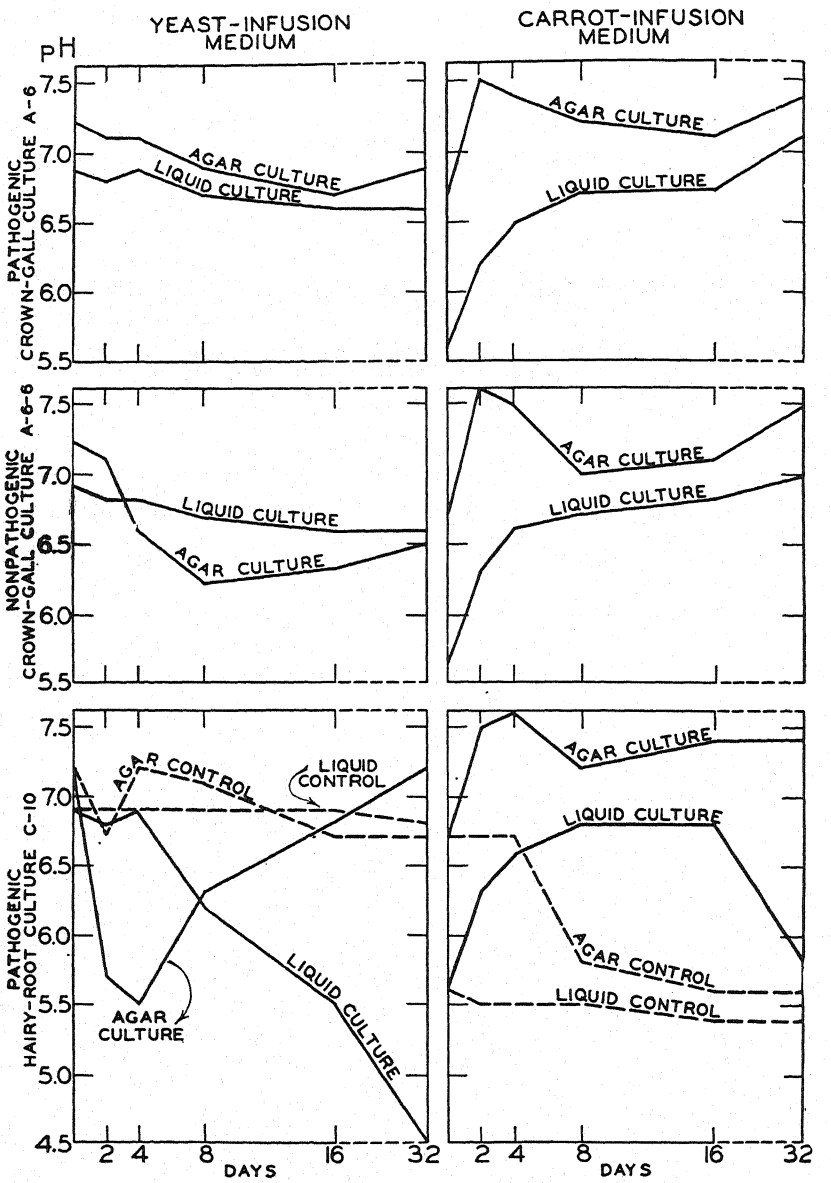


FIG. 1. Changes in pH value in both agar and liquid carrot-infusion and yeast-infusion glucose mineral-salts media, induced by the growth at 26° C. of pathogenic and nonpathogenic strains of the crown-gall organism and by that of a pathogenic strain of the hairy-root organism.

gradual fall in pH value, on agar media there was a very rapid fall followed by an almost equally rapid rise.

Readings on cultures on carrot-infusion media also showed differences in pH values induced in liquid and agar media. In liquid medium there was a gradual rise in pH value, often followed by a considerable fall in the cultures of the hairy-root organism. On agar medium there was a rapid rise in pH value in the cultures of all 3 organisms, followed by a slight drop towards the acid side. This latter phase was not constant in the cultures of the hairy-root organism, although it is shown to have occurred in the series illustrated in figure 1.

The data obtained on the changes in pH value induced in the agar substratum appear to bear out what might be expected from diffusion. In figure 2 is shown a typical graph drawn from data obtained in the manner

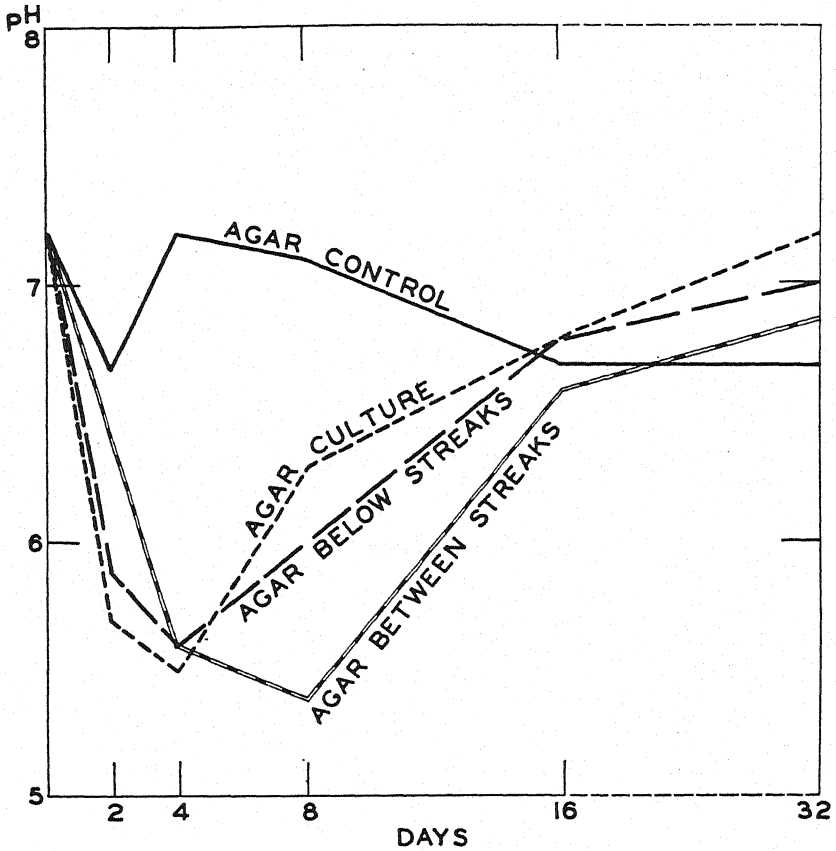


FIG. 2. The relation between the pH value induced in the bacterial growth and that induced in the agar substratum, below and midway between the streaks of growth. The graph illustrates data obtained from readings made on a culture of the hairy-root organism, growing at 26° C. on yeast-infusion glucose mineral-salts agar.

previously described. The pH value of the medium below the bacterial growth approximated that of the growth itself, though in most cases it showed a certain amount of lag. The pH value of the agar midway between the streaks showed a more marked lag.

In one of the preliminary series of readings, it was found that the control plates showed a marked rise in pH value instead of the normal gradual fall occasioned by the absorption of carbon dioxide. Qualitative tests with Nessler's reagent proved that this rise was caused by the production of large amounts of free ammonia by the plate cultures of the hairy-root organism. This free ammonia was produced in sufficient quantity to affect control plates stored in a separate metal container in the same incubator. In all series following this discovery, the hairy-root cultures were stored in a separate incubator.

The method employed in testing for ammonia did not permit the Nessler's reagent to come in contact with the medium. One drop of the reagent was placed on a microscope slide that was then laid on the surface of the baterial growth in the agar cultures. With the liquid cultures a similar slide was placed in an empty Petri dish into which was decanted a portion of the tube culture round the sides of the slide. In both cases readings were taken after the lapse of half an hour. The results of this investigation are presented in table 1. Adequate controls were maintained that showed both

TABLE 1.—*The production of free ammonia by a nonpathogenic and by a pathogenic strain of the crown-gall organism, and by the hairy-root organism, at 26° C. on liquid and agar carrot-infusion and yeast-infusion glucose mineral-salts media*

Media employed	Age of culture	Ammonia in culture employed[a]		
		Crown gall		Hairy-root
		Pathogenic	Non-pathogenic	Pathogenic
	Days			
Carrot-infusion liquid	2, 4, 8 and 16	+	+	+
" " "	32	0	0	+
Carrot-infusion agar	2, 4, 8, 16 and 32	0	0	+
Yeast-infusion etc. liquid	2	0	0	0
" " "	4, 8 and 16	0	0	+
" " "	32	0	0	0
Yeast-infusion etc. agar	2, 4, 8 and 16	0	0	+
" " "	32	0	0	0

[a] The presence of ammonia is indicated by +; its absence, by 0.

the sterile media and the air of the laboratory free from ammonia. From a study of the data it is seen that in both liquid and agar yeast-infusion and carrot-infusion media, the hairy-root organism produces free ammonia. In similar experiments with the crown-gall organism, no positive reaction to the test for ammonia was obtained, except in the carrot-infusion liquid medium. At the 32-day period, in those instances where free ammonia had previously been liberated, there was either a diminution in the strength of the reaction with the testing reagent, or else the test yielded a negative result.

DISCUSSION

Conner *et al.* (2) have shown that on yeast-infusion agar containing 1 per cent glucose, the hairy-root organism ferments most of the glucose within the first 10 days of growth; while in liquid medium, where the growth is not so rapid, the fermentation of the glucose is not completed in 30 days. They explain the absence of acids in the solid medium after the early period of growth by assuming that the pyruvic and acetic acids, formed by the fermentation of the glucose, have been either broken down or neutralized. These facts would account for the rapid fall followed by a rise in pH value, noted during the present work, in cultures of the hairy-root organism on yeast-infusion agar.

The slightly lower pH value induced by the growth of the non-pathogenic strain (A–6–6) of the crown-gall organism on yeast-infusion agar, while of doubtful significance in relation to pathogenicity, should be borne in mind as the only difference, revealed by this investigation, between the pathogenic and nonpathogenic strains. The results secured in these studies were similar at both 26° and 32° C., which temperatures are, respectively, below and above the critical maximum temperature range for gall formation (7). The temperature data secured, in addition to the slight difference noted between the pathogenic and nonpathogenic strains of the crown-gall organism, give no indication that pathogenicity is governed by the pH value induced.

The use of agar media in the present investigation has demonstrated that considerable variation in results may be obtained through the use of approximately similar media having different physical characteristics. This emphasises the dangers of comparing artificial and natural substrata.

The production of ammonia has an intimate bearing not only on changes in pH value, but also on the nitrogen metabolism involving the interrelationship of the host and parasite. Smith, Brown and McCulloch (11), Smith (10), Riker (7) and Riker *et al.* (8) reported the production of ammonia from liquid media by *Phytomonas tumefaciens*. In most of these cases the media contained peptone, which served as a source of ammonia

during fermentation. Riker *et al.* (8) also reported that *P. rhizogenes* produced ammonia from a peptone medium.

The rapid rise in pH value in cultures on carrot-infusion medium is, in all probability, caused by the formation of ammonia during the process of protein degradation. Several explanations of the subsequent fall in pH value, where such occurs, are suggested, including: (1) reduced ammonia production coupled with the formation of carbon dioxide or other acid substances from the medium; (2) utilization of the ammonia by the crown-gall organism. Sagen *et al.* demonstrated that of the crown-gall and hairy-root organisms, only the former is capable of utilising the ammonium radicle as a sole source of nitrogen. This fact, coupled with the data presented in this paper, shows clearly that a distinct difference exists between the nitrogen metabolisms of these two organisms.

SUMMARY

The changes in pH value induced by the hairy-root and by both pathogenic and nonpathogenic crown-gall bacteria on agar media were more rapid than those found in corresponding liquid media.

In yeast-infusion glucose mineral-salts agar medium, little change in reaction was brought about by the crown-gall organism, whereas the hairy-root bacterium produced a rapid fall in pH value followed by an almost equally rapid rise. In the corresponding liquid medium the growth was slower, resulting in a gradual fall in pH value throughout the 32-day period of the experiment. In carrot-infusion medium a rise in pH value occurred in cultures of all three organisms.

The nonpathogenic strain of the crown-gall organism differed from the pathogenic culture only by producing slightly more acid in the yeast-infusion glucose mineral-salts agar medium. No appreciable difference in pH value was noticed between cultures of the pathogenic and nonpathogenic strains of the crown-gall organism when they were grown, respectively, above and below the critical maximum temperature for gall formation.

It was found that the pH value of agar substrata, measured below and midway between the streaks of growth, followed that of the bacterial growth, with a lag that depended on the distance from the bacterial growth.

Free ammonia was formed on liquid and agar carrot-infusion and yeast-infusion glucose mineral-salts media by the hairy-root organism, and on liquid carrot-infusion medium alone by the crown-gall organism.

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EFFECT OF CERTAIN CHEMICALS ON THE "COMBINATION STREAK" VIRUS OF TOMATOES

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INTRODUCTION

Comparatively little has been done on the effect of chemicals on plant viruses. Plant pathologists lacked a satisfactory incentive for this type of research, as it seemed to promise little in the way of results that would be applicable to the control of virus diseases or very helpful in differentiating various viruses unless these could readily be obtained in a highly purified form. The most extensive work of this kind is that of Allard (1, 2), who tried a considerable number of substances on the tobacco-mosaic virus (presumably Johnson's virus no. 1). Johnson (14), himself, conducted some chemical trials with this virus and several other viruses causing various forms of tobacco mosaic, cucumber mosaic, spot necrosis, ring spot, and tomato stem necrosis. Fukushi (13), Lojkin and Vinson (19) and Caldwell (3) worked with tobacco mosaic and Doolittle (9) with cucumber mosaic. In these experiments over 80 substances were tested on the green tobacco mosaic, 8 on cucumber mosaic and only 1 or a few on each of the remainder.

However, with the exception of spot-necrosis experiments all the above-mentioned work dealt with simple viruses only. The field of virus complexes, particularly the possibility of their splitting into simpler component parts, was left practically untouched. The writer has taken up a study of the reaction of the so-called tomato "combination streak" virus to some of the most promising chemicals as far as the inactivation of either the entire complex or any of its portions is concerned. These studies have not been concluded, but as there is no prospect of their immediate resumption it is deemed justifiable to publish the results thus far obtained, as they may prove interesting and useful to other workers.

By "combination streak" is meant a naturally occurring complex virosis of tomatoes, which may be split into two component parts: (A) Green tobacco mosaic (Johnson's virus no. 1) and (B) latent potato mosaic, found in nearly all commercial stocks of American varieties of potatoes, but absent from healthy seedlings. It is thus designated in order to distinguish it from other forms of tomato streak, such as the dieback form of the Pacific Coast (apparently identical with English and Australian "spotted wilt"), experimental and glasshouse streaks described by English workers, two

[1] The writer feels greatly indebted to Dr. T. E. Rawlins, of the University of California, Berkeley, Calif., for his most helpful advice and suggestions as well as a quantity of valuable materials received from him in the course of this work.

simple streaks reported by Doolittle and Blood (10), and a number of unnamed forms now being studied by various investigators.

"Combination streak" becomes evident through necrotic lesions of various types on leaves, petioles, and stems, such as spots, streaks, and irregular blotches. It is accompanied by mosaic and crinkling of the leaves. When the leaf surface is mostly covered by these lesions the petiole is girdled, the affected leaves die, but acronecrosis, as a rule, is absent and the diseased plant continues to grow, although from the production viewpoint it is worthless. The usual method of isolating the component parts of the complex consists in the use of differential hosts, some of which are susceptible only to one form of mosaic. For the sake of brevity the combination form of streak will be referred to hereafter merely as streak, the green tobacco mosaic as mosaic A, and the latent mosaic as mosaic B. This use of the letters is identical with that employed by Samson.[2]

METHODS EMPLOYED

Fresh tomato plant material from plants affected with combination streak was crushed in a clean, dry food chopper and the juice was strained from the pulp through a piece of cheese cloth. No filtration or purification was attempted. The chemicals chosen for testing were first dissolved in distilled water. These solutions, as well as other liquid chemicals, were added to the strained juices to make up the desired concentrations. The mixtures were allowed to stand 2 hours before application to test plants. The control portions of the juice were mixed with the corresponding amounts of distilled water and also held for 2 hours before application. During this time all mixtures were shaken several times to insure homogeneity.

The inoculation tests were nearly all carried on under greenhouse conditions. Small tomato seedlings, averaging 5 to 6 inches in height, were used. At this age they are extremely susceptible to this form of streak. Check plants, inoculated with nontreated juices from tomato plants affected with combination streak, showed 100 per cent infection in each case and will be omitted from inclusion in the tables. All juices were brushed on the leaves with a camel-hair brush or a cotton swab. As a rule, symptoms of either streak or mosaic appeared 4 or 5 days later. It required 10 days or 2 weeks, however, for the symptoms to develop on some of the plants. If no symptoms appeared within 30 days, both viruses are considered permanently inactivated, or if one of the two mosaics failed to appear that virus was regarded as inactivated.

In selecting the chemicals for this work the principal attention was

[2] Samson, R. W. A study of certain viruses pathogenic to the tomato. Unpublished thesis, Purdue Univ., 1932.

given not to those that the streak virus may successfully resist, but to those to which it may readily succumb. Inasmuch as mosaic A enters as a component part of the combination streak, the previous work on this mosaic agent is of some interest in connection with the inactivation of the streak virus. However, the virus of mosaic B is by far more sensitive to destructive chemicals than is virus mosaic A; therefore, when a streak-containing juice is treated, weaker solutions tend to kill virus B and stronger solutions kill both A and B. There were a few exceptions in the writer's work that, *ipso facto,* appear particularly interesting.

MATERIALS USED AND RESULTS

Acids. Four acids were tested: Sulphurous, deoxycholic, boric, and diiodo-salicylic. Sulphurous acid was prepared by the action of warm dilute sulphuric acid on sodium sulphite (Na_2SO_3). The original strength, ascertained by the method outlined by Treadwell and Hall (23) was about 6 per cent. SO_2 is recognized as a powerful antiseptic employed successfully in industry. Trautwein (22) found H_2SO_3 effective in inactivating the foot and mouth virus. It was used by the writer for the first time in connection with the inactivation of plant viruses. Deoxycholic acid was extracted from ox-gall powder by the method described by White (24) without essential changes. Besides the final product two intermediate nonconjugated acids were also tried, namely: (a) A crystalline substance obtained after the first washing in acetic acid (prior to xylene purification) and containing some fatty acids (referred to in the table as xylene-cholic acid); and (b) a crystalline substance obtained after washing in xylene but before final washing in glacial acetic acid and containing some xylene (referred to in the table as fatty-cholic acid).

As may be seen from table 1, sulphurous acid (H_2SO_3) appeared to be highly toxic to the streak complex. At the concentration of about and above 1.5 per cent both component viruses were inactivated; below that and down to about 0.66 per cent the virus of mosaic B was completely eliminated without injury to the virus of mosaic A. With still lower concentrations the effect was only partial or none at all.

Deoxycholic acid also showed a distinct toxicity, although not so phenomenal as in the case of pneumococcus, as found by Downie, Stent, and White (11). Only the 5 per cent strength of this chemical appeared to be totally fatal to mosaic B and nearly so to mosaic A. The two intermediate substances gave approximately the same results. This is the first application of deoxycholic acid to the plant-virus-inactivation work. In no case was mosaic B left active after the elimination of mosaic A. Boric acid (H_3BO_3), in the concentrations used, proved entirely ineffective. Five per

cent solution of diiodo-salicylic acid showed some toxic effect comparable with that produced by intermediary bile acids.

TABLE 1.—*Results of treating streak-infective tomato juices with acids*

Concentration of chemicals	Number of plants				
	Inoculated	Streak	Mosaic A only	Mosaic B only	Healthy
Per cent					
H_2SO_3					
0.18	6	All	0	0	0
0.36	6	1	4	0	1
0.65	6	0	2	0	4
0.72	6	0	4	0	2
1.3	6	0	4	0	2
1.5	6	0	0	0	All
2.65	6	0	0	0	"
3.0	6	0	0	0	"
Deoxycholic acid					
0.06	4	All	0	0	0
0.12	4	"	0	0	0
0.25	4	"	0	0	0
0.5	4	"	0	0	0
1.0	4	"	0	0	0
2.0	4	3	1	0	0
5.0	4	0	1	0	3
Xylene-cholic acid					
0.1	4	All	0	0	0
1.0	4	"	0	0	0
5.0	4	1	1	0	2
Fatty-cholic acid					
0.1	4	All	0	0	0
1.0	4	"	0	0	0
5.0	4	1	1	0	2
H_3BO_3 (Boric acid)					
2.5 and 5.0	22	All	0	0	0
Diiodo-salicylic acid ($I_2C_6H_2COOH$					
5.0	8	1	5	0	2

Sulphites. Sodium sulphite (Na_2SO_3), sodium acid sulphite ($NaHSO_3$), and potassium metabisulphite ($K_2S_2O_5$) were used. All three were employed for the first time as inactivators in the plant-virus work. None of these salts was nearly so effective as H_2SO_3. The virus of mosaic B was present to a greater or lesser degree in all the salt and plant-juice mixtures containing 2.5 and 5.0 per cent of these sulphites, whereas the virus of mosaic A showed also a partial survival, even in a 10 per cent mixture of $K_2S_2O_5$. Summarized results are given in table 2.

TABLE 2.—*Results of treating streak-infective tomato juices with certain sulphites*

Concentration of chemicals	Number of plants				
	Inoculated	Streak	Mosaic A only	Mosaic B only	Healthy
Per cent					
Na_2SO_3					
2.5	6	All	0	0	0
5.0	6	"	0	0	0
$NaHSO_3$					
2.5	6	All	0	0	0
5.0	6	2	2	0	2
$K_2S_2O_5$					
2.5	6	5	1	0	0
2.5	6	All	0	0	0
5.0	6	2	2	0	2
10.0	6	0	2	0	4

Sulphates. A few sulphates were previously used by other workers in connection with the virus-disease work. Thus, Allard (2) used copper sulphate on tobacco-mosaic and inactivated it in a 0.2 per cent mixture. The same virus was treated by him with manganese sulphate at the strength of approximately 7.5 per cent, but the results were negative. $CuSO_4$ also was used by Doolittle (9) on cucumber mosaic, which was inactivated at 0.5 per cent. Caldwell (3) tried mercuric sulphate in acid solution in connection with tobacco mosaic and obtained inactivation, but the virus was reactivated on addition of alkali. Since tobacco mosaic (mosaic A) is a part of combination-streak complex, Allard's tests with $CuSO_4$ are to be especially noted here. In the writer's treatments of the streak-containing juice with this chemical, 2.5 and 5.0 per cent strengths were used. In all cases mosaic B was eliminated and in most cases mosaic A, also. A similar lytic action was manifested by $KHSO_4$ with which a 0.6 per cent mixture alone was not entirely effective against mosaic B, whereas mosaic A showed some activity even after the 5 per cent bath. On the other hand, K_2SO_4 at similar dilutions was entirely ineffective against any part of the complex. The most decisive inactivating action was shown by antimony sulphate and bismuth sulphate, although only one controlled experiment in each case was performed. In these experiments the streak complex was entirely inactivated by a 5 per cent solution of these salts. Field experiments also were carried on with these substances, but the plants were unprotected and naturally became contaminated. Therefore, they do not show a complete eradication of the streak complex nor of the mosaic A virus, although the effectiveness of these chemicals is clearly revealed. Sodium thiosulphate ($Na_2S_2O_3$) showed very weak lytic action.

TABLE 3.—*Results of treating streak-infective tomato juices with certain sulphates*

Concentration of chemicals	Number of plants				
	Inoculated	Streak	Mosaic A only	Mosaic B only	Healthy
Per cent					
$CuSO_4$					
2.5	6	0	1	0	5
5.0	17	0	3	0	14
$KHSO_4$					
0.6	6	1	5	0	0
1.25	6	0	5	0	1
2.5	6	0	6	0	0
5.0	6	0	2	0	4
5.0	6	0	0	0	All
K_2SO_4					
5.0	6	All	0	0	0
6.3	6	"	0	0	0
$Sb_2(SO_4)_3$					
Grhs.:					
5.0	13	0	0	0	All
Field:					
8.0	11	0	1	0	10
Half-saturated	12	1	8	0	3
Saturated	10	0	3	0	7
$Bi_2(SO_4)_3$					
Grhs.:					
5.0	5	0	0	0	All
Field:					
8.0	11	2	8	0	1
Half-saturated	10	6	3	0	1
Saturated	10	2	8	0	0
$Na_2S_2O_3$					
0.01– 1.0	9	All	0	0	0
5.0	4	"	0	0	0
10.0	6	"	0	0	0
15.0	6	"	0	0	0
20.0	6	4	2	0	0
25.0	6	3	2	0	1
$CoSO_4$					
5.0	14	6	1	3	4
$NiSO_4$					
5.0	6	2	0	4	0
10.0	6	3	0	3	0
10.0	6	All	0	0	0
15.0	6	2	4	0	0
20.0	6	0	2	0	4
20.0	6	1	0	2	3
25.0	6	5	0	0	1
25.0	6	2	2	0	2
$ZnSO_4$					
Grhs.:					
2.5	26	23	1	0	2
5.0	11	7	2	0	2
10.0	11	3	1	0	7
Field:					
2.5	10	6	1	0	3
5.0	15	5	6	2	2
10.0	13	0	2	1	10

Rather unexpected results were obtained with cobalt sulphate, nickel sulphate, and zinc sulphate. Their lytic effect, unlike that of other tested substances, was not much stronger with mosaic B than with mosaic A. When the solution was not strong enough to kill both components parts, either one or the other would survive. The results obtained with these sulphates to date, while of considerable interest, do not warrant a definite conclusion as to the effect of the chemicals in question, and merely suggest a possibility of splitting viruses A and B in the combination streak complex by chemical means. These results, however, cannot yet be predicted. It is possible that in the case of $NiSO_4$ the separation of the B virus was due to cobalt present as an impurity in the amount of 0.008 of 1 per cent. The experimental data are summarized in table 3. Allard (1) successfully used nickel hydroxide to inactivate tobacco mosaic, but no other nickel compounds were employed.

Bile and bile salts. Bile was used successfully by Friedberger and Yamamoto (12) for treatment of variola, by Landsteiner *et al.* (15) for poliomyelitis, and by Todd (21) for cattle plague. Apparently, the writer applied it to plant-virus-inactivation work for the first time. In these experiments it did not prove to be very toxic to the streak complex. Virus B showed some activity, even in the 20 per cent solution, and virus A in the 25 per cent solution. No healthy plant was obtained at or below 15 per cent concentration. Sodium taurocholate and sodium glycocholate were used only in 2.5 and 5.0 per cent concentrations, which showed no effect on either of the two component parts of streak (table 4). Previously, Allard (2) found that a 5-day exposure to 5 per cent sodium taurocholate solution inactivated tobacco virus, but not the exposure to 2.0 per cent solution for the same period of time. No report is known as to the use of sodium glycocholate.

Iodine. Chandler (4, 5, 6, 7) and Chandler and Miller (8) in Michigan obtained very striking results in treating blackhead in turkey with colloidal iodine, also known on the market as iodine suspensoid. This form of iodine appeared to be the most active and available form of this element. Prof. Chandler very kindly arranged with Merck and Company to supply the writer with iodine suspensoid for his experiments. It showed a decided toxicity and was lytic to mosaic B in a 1.0 per cent solution and to both component viruses in a 5.0 per cent solution. A 1.0 per cent solution of KI, however, used at the same time, showed no effect either on the virus of mosaic A or on the virus of mosaic B (table 5).

DYES, DISINFECTANTS, AND MISCELLANEOUS MATERIALS

In the work of Lewis (16, 17, 18) with various animal and fowl viruses and of Schultz and Kruger (20) with Staphylococcus bacteriophage,

TABLE 4.—*Effect of bile and bile salts on the streak virus*

Concentration of chemicals	Number of plants				
	Inoculated	Streak	Mosaic A only	Mosaic B only	Healthy
Per cent					
Bile					
0.01– 1.0	9	All	0	0	0
2.5	4	"	0	0	0
5.0	4	3	1	0	0
10.0	6	All	0	0	0
10.0	6	3	3	0	0
15.0	6	2	4	0	0
20.0	6	2	2	0	2
25.0	6	0	5	0	1
Sodium taurocholate					
2.5	6	All	0	0	0
5.0	6	"	0	0	0
Sodium glycocholate					
2.5	6	All	0	0	0
5.0	6	"	0	0	0

methylene blue proved to be a very satisfactory inactivator even at very low concentrations. The writer made a test of a 1 per cent concentration on 3 young plants, but the results were negative. No other dyes were employed in this streak work. In the disinfectant group, mercurochrome was ineffective at and below 1.0 per cent strength, whereas lysol at 2.5 per cent showed some effect on mosaic B. Of other substances used in the concen-

TABLE 5.—*Effect of iodine preparations on the streak virus*

Concentration of chemicals	Number of plants				
	Inoculated	Streak	Mosaic A only	Mosaic B only	Healthy
Per cent					
Iodine suspensoid					
0.1	4	All	0	0	0
0.2	4	"	0	0	0
0.5	4	"	0	0	0
1.0	4	0	4	0	0
2.0	4	0	3	0	1
5.0	4	0	0	0	All
10.0	4	0	0	0	"
10.0	6	1	0	0	5
10.0	6	0	0	0	All
10.0	6	0	0	0	"
25.0	6	0	0	0	"
50.0	6	0	0	0	"
KI					
1.0	4	All	0	0	0

trations not exceeding 5 per cent, saponin, rotenone, thiourea and cholesterol, proved to be entirely ineffective. One plant out of 12 infected with the juice containing 10 per cent of reuniol entirely escaped infection, which may, however, be accidental. A 5 per cent solution of sodium ricinolate, on the other hand, showed a definite, though moderate, effect on both mosaic A and mosaic B.

Another salt, sodium salicylate, included in this group, showed a strong toxicity to the entire streak complex in the 5 and 10 per cent mixtures and some effect on mosaic B, even at 1 per cent. Further details are given in table 6.

TABLE 6.—*Effect of certain chemicals on the streak virus*

Concentration of chemicals	Number of plants				
	Inoculated	Streak	Mosaic A only	Mosaic B only	Healthy
Per cent					
Methylene blue					
1.0	3	All	0	0	0
Mercurochrome					
0.01–1.0	9	All	0	0	0
Lysol					
2.5	6	5	1	0	0
2.5	6	3	3	0	0
Saponin					
0.01–1.0	9	All	0	0	0
5.0	4	"	0	0	0
Rotenone					
5.0	12	All	0	0	0
Thiourea					
5.0	12	All	0	0	0
Cholesterol					
5.0	12	All	0	0	0
Reuniol (emulsion)					
10.0	12	11	0	0	1
Sodium ricinolate					
5.0	10	6	1	0	3
Sodium salicylate ($NaC_7H_5O_3$)					
1.0	4	3	1	0	0
5.0	4	0	0	0	All
10.0	4	0	0	0	"

SUMMARY

A number of chemical substances, known to produce strong lytic action on certain microorganisms, were added to unfiltered tomato juices containing the virus of combination streak. After allowing them to remain in these solutions for 2 hours, these juices were applied to young healthy tomato plants just as were untreated juices. The results were evaluated in comparison with the control plants, which in every case showed 100 per cent infection in from 4 to 5 days. The virus was considered inactivated when symptoms failed to appear within 30 days.

According to the results obtained, the chemicals used in this work fall into 3 groups: (a) Those showing little or no effect on either of the two component parts of the streak; (b) those having a decided lytic effect on both components with virus B, as a rule, being the weaker part of the complex; and (c) those that revealed no special affinity for either virus A or virus B.

The first group, in the order of ascending degree of effectiveness, was comprised of the bile and the 2 bile salts, $I_2C_6H_3COOH$, $Na_2S_2O_3$, K_2SO_4, Na_2SO_3, $K_2S_2O_5$, H_3BO_3, saponin, rotenone, thiourea, cholesterol, reuniol, and sodium ricinolate. Methylene blue, mercurochrome, and lysol may belong to this group, although these substances were tested only in weaker dilutions.

The second group, using the same order, consisted of the unconjugated bile acids, $CuSO_4$, $KHSO_4$, $NaC_7H_5O_3$, iodine suspensoid, $Sb_2(SO_4)_3$, $Bi_2(SO_4)_3$ and H_2SO_3. The latter was effective in relatively low concentrations.

The remaining tested chemicals, namely, $CoSO_4$, $NiSO_4$, and $ZnSO_4$, especially the first two, showed a lytic effect on both components of the streak complex but, unlike other substances tested, this effect was little stronger on the virus of mosaic B than on that of mosaic A, since, when the solution was not strong enough to kill both viruses, sometimes one and sometimes the other would survive. The data, however, are not sufficient to predict the results of such treatments.

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THE INHERITANCE OF RESISTANCE TO COMMON BEAN MOSAIC IN FIELD AND GARDEN BEANS[1]

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An investigation of the mode of inheritance of mosaic resistance was undertaken coincidental with the development of 2 mosaic-resistant varieties of Refugee type beans. These resistant varieties of Refugee, Wisconsin Refugee, and Idaho Refugee, were developed by Pierce and Walker (7) from crosses of the regular Refugee Green variety with the resistant variety Corbett Refugee.

It was noted that in these crosses the F_1 hybrids were resistant to mosaic. McRostie (2) had previously found that in various crosses in which the Robust variety was used as the resistant parent, susceptibility was at least partially dominant. Also, Parker and Brink (5) in studying Robust-Refugee Green crosses, found that in F_2 progenies 56 per cent of the plants were resistant in crosses in which Robust was used as the female parent and that only 1.3 per cent of the plants were resistant after the reciprocal cross. It was apparent, therefore, that in all probability the resistances of Corbett Refugee and Robust were inherited differently and that the whole problem needed further study. In the present investigation the inheritance of mosaic resistance of 3 resistant varieties, Corbett Refugee, Great Northern UI No. 1, and Robust was studied in crosses with the susceptible Refugee Green variety. This paper presents the results obtained from an analysis of the F_1 and F_2 generations of these crosses.

EXPERIMENTAL MATERIALS AND METHODS

The following crosses were made in the greenhouse at Madison, Wisconsin, beginning in the fall of 1930:

(R)[3] Corbett Refugee[4] × (S) Refugee Green (and reciprocal).

(R) Great Northern UI No. 1 × (S) Refugee Green (and reciprocal).

(R) Robust × (S) Refugee Green.

(R) Corbett Refugee × (R) Great Northern UI No. 1 (and reciprocal).

[1] Published with the approval of the Director of the Idaho Agricultural Experiment Station as Research Paper No. 127.

[2] The author gratefully acknowledges his indebtedness to Dr. J. C. Walker, under whose direction the problem was initiated, for his interest and encouragement throughout the course of the investigation; and to Dr. C. W. Hungerford for counsel during the later phases of the study and preparation of the manuscript.

[3] (R) designates variety resistant to common bean mosaic.
(S) designates susceptible variety.

[4] The female parent is written first and the male parent second in all crosses.

(R) Corbett Refugee × (R) Robust.
(R) Great Northern UI No. 1 × (R) Robust.

These crosses were made on greenhouse-grown, mosaic-free plants. The 4 varieties used were among those tested (6) for resistance and susceptibility to common bean mosaic. Greenhouse inoculation trials had shown that these varieties were either homozygous for resistance or for susceptibility. Especial mention is due Mr. R. D. Corbett who developed the Corbett Refugee variety from a single mosaic resistant plant found in a heavily infected stand of Refugee Green beans. He very kindly furnished seed for this investigation. In figure 1 is shown the mosaic resistant Corbett Refugee variety and



FIG. 1. Field plantings of two bean varieties used in various hybrids. Mosaic-resistant Corbett Refugee variety at left. Mosaic-infected Refugee Green variety at right.

the mosaic-infected Refugee Green variety grown in the field trials in 1934.

The bean mosaic virus used was the common bean mosaic virus (*Bean virus 1*) originally described by Reddick and Stewart (8, 9, 10) and shown by them to be seed-borne and to be nontransmissible to the Robust variety. The susceptibility of the F_1 and F_2 progenies to this virus was determined in the field and greenhouse at the University of Wisconsin, during 1931 and 1932, and at the University of Idaho during 1933 and 1934. All progenies were artificially inoculated by rubbing the young primary leaves with infected leaf material. The field trials were, in addition, subject to infection from natural sources.

It was previously shown (6) that the yellow bean-mosaic virus (*Bean virus 2*), which may overwinter on sweet clover, will affect many varieties of the common bean, including varieties resistant to the common form of bean mosaic. Since the field symptoms of both types of mosaic are readily confused, it was imperative that natural infections with the yellow form be

ruled out. This was done by making transfer inoculations from plants suspected of being affected with *Bean virus 2* to differential varieties in the greenhouse. In this manner natural infections with yellow bean mosaic were effectively determined and not confused with infections of common bean mosaic.

Also, it was found that certain chlorophyll-deficient segregants of the type described by Burkholder and Muller (1) and by Parker (3, 4) might readily be confused with mosaic symptoms. It was felt necessary, therefore, to test plants of this type by making transfer inoculations to known susceptible plants in order to determine whether or not they were actually infected with mosaic. This added considerably to the task of classifying progenies; but the actual testing showed its necessity, for it was found that while some chlorophyll-deficient plants were resistant, others were actually infected with mosaic but with the symptoms so masked that the infection was impossible of detection without an actual test.

The F_2 tests were made only with seed obtained from F_1 plants that had been tested for mosaic reaction. Thus in crosses found to be susceptible in F_1, there were a certain number of plants showing primary infection in F_2. Those F_2 plants arising from mosaic-infected seed were of necessity classified as susceptible plants.

Seed of all progenies was scarified before planting to insure uniform and complete germination. Inoculations were made as soon as the young primary leaves were fully expanded. As soon as symptoms appeared in the F_2 progenies, the infected plants were counted and removed; the remaining healthy plants were then reinoculated so as to insure the infection of all susceptible individuals. Plants were removed as soon as they showed unmistakable symptoms in order to insure accuracy in classification, since the mosaic symptoms tend to become masked as the plants grow older.

EXPERIMENTAL RESULTS

F_1 Progeny Tests

The F_1 generation of the crosses listed in table 1 were all grown in the field and were artificially inoculated with the virus of common bean mosaic besides being subject to infection from natural sources. Crosses in which Corbett Refugee was involved were resistant to mosaic in the F_1 generation. Thus hybrids of (R) Corbett Refugee with (S) Refugee Green, with (R) Great Northern UI No. 1 and with (R) Robust were all resistant. Also the cross, Great Northern UI No. 1 × Robust was resistant. However, hybrids of (R) Robust and (R) Great Northern UI No. 1 with (S) Refugee Green were found to be susceptible. There was no apparent difference in susceptibility to mosaic of reciprocal crosses involving the same parents in the first generation.

TABLE 1.—*Occurrence of common bean mosaic in the F_1 and F_2 progenies of certain reciprocal bean hybrids when inoculated with the virus of common bean mosaic*

Parentage of crosses[a]	Total F_1 plants tested	F_1 mosaic reaction	Segregation in F_2 Resistant: Susceptible	Resistant F_2 plants
				Per cent
(S)[b] Refugee Green No. 109 × (R) Corbett Refugee	31	resistant	1688: 361	82.3
(S) Refugee Green No. 108 × (R) " "	6	"	278: 69	80.1
(S) Refugee Green No. 114 × (R) " "	11	"	275: 66	80.6
(S) Refugee Green No. 112 × (R) " "	7	"	198: 32	86.1
TOTALS	55	resistant	2439: 528	82.2
(R) Corbett Refugee × (S) Refugee Green No. 109	40	resistant	2111: 262	88.9
(R) " " × (S) Refugee Green No. 108	3	"	115: 15	88.4
(R) " " × (S) Refugee Green No. 114	5	"	89: 11	89.0
(R) " " × (S) Refugee Green No. 112	3	"	91: 15	85.8
TOTALS	51	resistant	2406: 303	88.8
(S) Refugee Green No. 109 × (R) Grt. Northern UI No. 1	19	susceptible	57: 316	15.2
(R) Grt. Northern UI No. 1 × (S) Refugee Green No. 109	9	"	82: 375	17.9
(R) Robust × (S) Refugee Green No. 109	3	susceptible	82: 598	12.0
(R) Great Northern UI No. 1 × (R) Robust	4	resistant	59: 0	100.0
(R) Corbett Refugee × (R) Grt. Northern UI No. 1	7	"	47: 6	88.6
(R) Grt. Northern UI No. 1 × (R) Corbett Refugee	16	"	141: 16	89.8
(R) Corbett Refugee × (R) Robust	8	"	95: 25	79.2
(R) Idaho Refugee × (S) Refugee Green No. 109	1	"	81: 13	85.9
(S) Refugee Green No. 109 × (R) Idaho Refugee	2	"	91: 13	87.5
(R) Wisconsin Refugee × (S) Refugee Green No. 109	2	"	159: 28	85.0

[a] The female parent is written first and the male parent second in all crosses.
[b] S designates variety susceptible to common bean mosaic.
R designates resistant variety.

The fact that Corbett Refugee gave a different reaction in F_1 in crosses with Refugee Green than did Robust or Great Northern UI No. 1 with Refugee Green, is alone sufficient evidence to indicate that Corbett Refugee possesses a different type or degree of resistance. Further evidence, however, is to be found by an examination of the F_2 results.

F_2 Progeny Tests

Corbett Refugee × Refugee Green. As shown in table 1, Corbett Refugee was crossed with 4 different susceptible strains of Refugee Green. Offspring of crosses of Corbett Refugee and Refugee Green strain No. 109 contained 6.6 per cent more resistant plants when the susceptible variety (Refugee Green) was used as the pollen (♂) parent than when used as the pistillate (♀) parent. Refugee Green strains No. 108 and No. 114 gave similar results. There was no significant difference in reciprocal crosses of the Refugee Green strain No. 112 with Corbett Refugee.

A summary of the data of all 4 strains of the cross Refugee Green (♀) × Corbett Refugee (♂) shows that 2439 individuals were classified as resistant and 528 as susceptible. The expectation on the basis of a 13 : 3 ratio would be 2410.7 resistant to 556.3 susceptible. However, a summary of the data on the reciprocals of these crosses shows 2406 classified as resistant and 303 as susceptible, which is too far from a 13 : 3 expectancy to be significant. It is possible that certain cytoplasmic elements may in some manner have suppressed expression of the susceptibility of 1/16 of the population in the latter case.

In view of the fact that there is a difference of 6.6 per cent resistance between reciprocal crosses, suggesting that the inheritance of resistance may not be entirely Mendelian, no factorial explanation for the results secured is attempted.

Great Northern UI No. 1 × Refugee Green. An analysis of the F_2 generations of reciprocal crosses of Great Northern UI No. 1 and Refugee Green shows that from 15 to 18 per cent of the plants were resistant (Table 1). Figure 2 shows the relative number of mosaic-resistant plants obtained in the F_2 generation of this cross compared to a cross of Great Northern UI No. 1 with Corbett Refugee. The 15 to 18 per cent resistant plants obtained in the Great Northern UI No. 1–Refugee Green cross is much lower than the 82 to 89 per cent found in the Corbett Refugee crosses with Refugee Green. This result, however, was to be expected, since the Great Northern UI No. 1 hybrids were found susceptible in F_1 and the Corbett Refugee hybrids resistant. The reciprocals of the Great Northern UI No. 1–Refugee Green cross show a divergence of only 2.7 per cent resistant plants.

Robust × Refugee Green. A cross of Robust × Refugee Green No. 109 in which Robust was used as the female parent has been analyzed. As shown

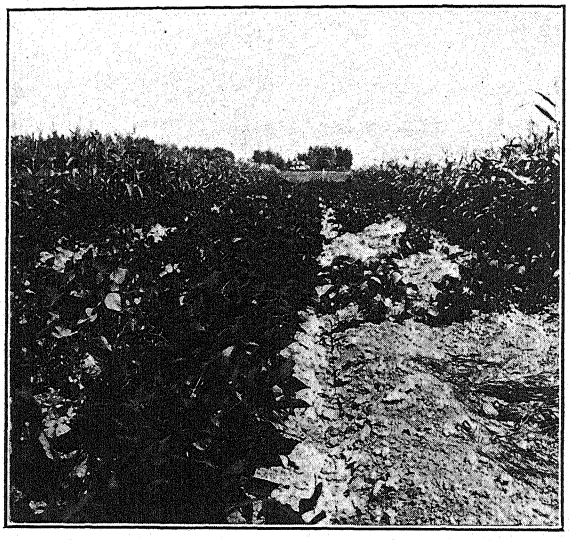


FIG. 2. Relative number of mosaic-resistant plants obtained in F_2 generation of certain crosses. The susceptible plants had been removed. Great Northern UI No. 1 × Corbett Refugee at left. Great Northern UI No. 1 × Refugee Green at right. Note that relatively few plants were resistant.

in table 1, an F_2 population of 680 plants was found to contain only 82 resistant plants or 12 per cent. The reciprocal cross was not studied. However, even if no resistant plants were to be obtained in the reciprocal, there could not be so wide a difference between reciprocal crosses as was reported by Parker and Brink (5). In a similar cross they found 56 per cent resistant plants in F_2 when Robust was used as the female parent and only 1.3 per cent after the reciprocal cross. Obviously, the data are somewhat conflicting; but it is possible that the strain of Robust or strain of Refugee Green used in the 2 investigations differed genetically. However, as shown in the results obtained with the Corbett Refugee–Refugee Green crosses, there was little observable difference among the 4 different strains of Refugee Green used in this investigation.

Crosses Between Resistant Varieties. It is interesting to note that segregation of susceptible individuals was obtained in the F_2 generation of crosses between certain resistant varieties (Table 1). Corbett Refugee × Great Northern UI No. 1, both resistant varieties, segregated between 10 and 12 per cent susceptible individuals in F_2 with no appreciable difference in reciprocal crosses (Figure 2, Left). The percentage of susceptible individuals segregating in this cross is practically the same as in the cross of Corbett Refugee (♀) × Refugee Green (♂). It is probable that the resistant

Great Northern UI No. 1 variety carries factors for susceptibility as well as for resistance.

Also, a Corbett Refugee × Robust cross segregated susceptible individuals in F_2. In an F_2 population of 120 individuals 25 were found susceptible (Table 1). This result might well have been predicted from the results obtained in the crosses of Robust × Refugee Green, Great Northern UI No. 1 × Refugee Green, and Corbett Refugee × Great Northern UI No. 1. That is, since Robust and Great Northern UI No. 1 behaved similarly in crosses with Refugee Green, it would be expected that Robust–Corbett Refugee crosses would segregate similarly to the Great Northern UI No. 1–Corbett Refugee cross. The probability that the resistances of Robust and Great Northern UI No. 1 are similar is further indicated by the results of a cross of these 2 varieties. A small progeny of 59 individuals of a Robust–Great Northern UI No. 1 cross did not segregate any susceptible individuals (Table 1).

However, it should be pointed out that Great Northern UI No. 1 appears to have a slightly higher degree of resistance, as evidenced by other crosses, than does Robust. Thus, the F_2 of the Great Northern UI No. 1 × Refugee Green cross segregated 17.9 per cent resistant individuals, while Robust × Refugee Green segregated only 12 per cent resistant individuals. And furthermore, the crosses of Robust and Great Northern UI No. 1 with Corbett Refugee, in which the Robust crosses segregated 20.8 per cent susceptible plants, and the Great Northern UI No. 1 crosses segregated 11.4 per cent, indicate that Robust is slightly less resistant genetically.

These differences are not great, especially when compared to the high degree of resistance of Corbett Refugee, and since the cross of Great Northern UI No. 1 × Robust failed to segregate, it would appear that any difference in the resistances of these two varieties might well be attributed to modifying factors.

Wisconsin Refugee and Idaho Refugee × Refugee Green. Pierce and Walker (7) demonstrated the practicability of using Corbett Refugee as a parent in the development of mosaic-resistant Refugee beans. The 2 varieties, Wisconsin Refugee and Idaho Refugee, which they developed from the cross of Refugee Green (♀) with Corbett Refugee (♂), were used in back crosses to Refugee Green. The F_2 segregation shown in table 1 indicates that the 2 new varieties possess approximately the same degree of resistance as the original resistant parental variety, Corbett Refugee.

This is evidence that, even though a slightly less number of resistant plants were obtained in the original Refugee Green (♀) × Corbett Refugee (♂) cross than in the reciprocal, it was still possible to obtain F_2 plants with the same degree of resistance as the resistant pollen parent.

DISCUSSION

The significance of the present investigations is largely in showing that certain mosaic resistant varieties of the common bean possess a different type or degree of resistance, and that it is possible to predict with a reasonable degree of accuracy the results that may be expected from crosses of these various resistant varieties with susceptible types.

Other than the slight difference found in the reaction of certain reciprocal crosses, the results on the whole were quite consistent. Thus, Great Northern UI No. 1, showed a tendency to give slightly more resistant individuals in F_2 than did Robust, both in crosses with Refugee Green and with Corbett Refugee. Furthermore, Corbett Refugee has consistently demonstrated a dominant type of resistance, as shown by resistant F_1 hybrids followed by a high percentage of resistant plants in F_2 populations.

It is believed that the results are sufficiently consistent to allow for a fairly accurate prediction of what may be expected of these resistant varieties in crosses with susceptibile types. This is, after all, the primary value of a genetical study insofar as the practical plant breeder is concerned, regardless of whether or not a strict factorial explanation is advanced. No explanation on a factorial basis is attempted because of the difference obtained in certain reciprocal crosses, which suggests that the inheritance of resistance may in part be non-Mendelian. However, since the differences in F_2 were found to be relatively slight, and since F_1 was not matroclinous, we are not inclined to agree wholly with the suggestion of Parker and Brink (5) that the basis of resistance is something probably carried in the cytoplasm. It would appear from our results that more probably specific genes are responsible for resistance, and that they may be influenced somewhat by the cytoplasm with which or within which they are acting.

SUMMARY

The studies reported in this paper were concerned with the inheritance of resistance to common bean mosaic in crosses involving three resistant varieties, Corbett Refugee, Robust, and Great Northern UI No. 1, and one susceptible variety, Refugee Green.

All hybrids involving Corbett Refugee were found to be resistant to common bean mosaic in the F_1 generation. The F_1 generation of crosses of Great Northern UI No. 1 and Robust with Refugee Green were susceptible.

In the F_2 generation the Corbett Refugee–Refugee Green hybrids segregated 89 per cent resistant plants when Corbett Refugee was used as the male (pollen) parent and 82 per cent after the reciprocal cross. The F_2 progenies of Great Northern UI No. 1 × Refugee Green segregated 15 to 18 per cent

resistant plants, and Robust × Refugee Green segregated 12 per cent resistant plants.

Crosses between certain resistant varieties were found to segregate susceptible plants in F_2. Corbett Refugee × Great Northern UI No. 1 segregated 11 per cent susceptible plants; and Corbett Refugee × Robust segregated 20.8 per cent susceptible plants. Great Northern UI No. 1 × Robust did not segregate in F_2, indicating that the resistances of these two varieties were similar. Two homozygous resistant varieties, Idaho Refugee and Wisconsin Refugee, selected from the Refugee Green (♀) × Corbett Refugee (♂) cross, were backcrossed to Refugee Green. An F_2 analysis showed that these 2 resistant varieties possessed the same degree of resistance as the original resistant parent variety, Corbett Refugee.

It was concluded that the data obtained from the various crosses were consistent enough to allow for fairly accurate predictions of what might be expected of the resistant varieties studied when crossed with other varieties.

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PHYTOPATHOLOGICAL NOTES

Areolate Spot of Citrus Caused by Leptosphaeria bondari.—The known range of areolate spot, a striking leaf and twig disease of citrus (*Citrus* spp.), so named and discussed by Bondar[1] as occurring in Bahia, Brazil, has recently been extended to Dutch Guiana and Venezuela. This information is based upon specimens at hand (Fig. 1, A and B) from Paramaribo, Surinam, Dutch Guiana (C. Stahel, Oct. 7, 1933), and from San Cristobal, Táchira, Venezuela (I. J. Otera, Oct. 28, 1933), sent by C. Chardon and Stahel, respectively to H. S. Fawcett, from whom they were received as then unidentified. In southern Brazil records of the disease in the Federal District and São Paulo were recently reported,[2] and during the past year specimens of diseased leaves have been received from Morretes, Paraná (H. Grillo, Feb. 15, 1934).

Areolate spot, which is particularly destructive in the nursery, is apparently a disease of moist warm climates. Both Bondar and Stahel[3] refer to it as prevalent in the nursery during the rainy season. Stahel[3] wrote that during this period nursery plants become entirely defoliated, but that they recover during the dry season. The disease has not been observed in the plantations in the highlands of São Paulo, where most of the citrus groves are located.

On the lesions of areolate spot, the symptoms of which are described by Bondar, he found 3 forms of what he interpreted to be the same fungus, 2 pycnidial, one with conidia 2–3 $\mu \times 4\ \mu$, and the other with 4-septate, hyaline conidia 20 μ in length, and a Leptosphaeria. He does not state the color of the smaller conidia, but those observed in the specimens examined, including those from Bondar, are hyaline, becoming distinctly colored (Fig. 1, C and E). This stage, therefore, may be considered a Coniothyrium, which genus generally is recognized as the conidial stage of Leptosphaeria. At São Paulo monosporous cultures were made from these 2 stages as present on a specimen contributed by Bondar (Nov. 1932, Bondar 337) on which only these 2 stages were found. The 2 isolations were of similar appearance; Hendersonia, or what is possibly the septate conidial form referred to by Bondar, was obtained from the ascospore culture, while the conidial culture yielded only Coniothyrium. Inoculations were made with both cultures, but no definite results were obtained.

[1] Bondar, G. Insectos daminhos e molestias da laranjeira no Brasil. Bol. Lab. Path. Veg. Bahia, 7, 79 pp. 1929.

[2] Bitancourt, A., J. P. Fonseca, and M. Autuori. Manual de Citricultura, II Parte. Doencas, pragas e tratamentos. 212 p. Edição da "Chacaras e quintaes," São Paulo. 1933.

[3] Letter to H. S. Fawcett, dated Oct. 7, 1933.

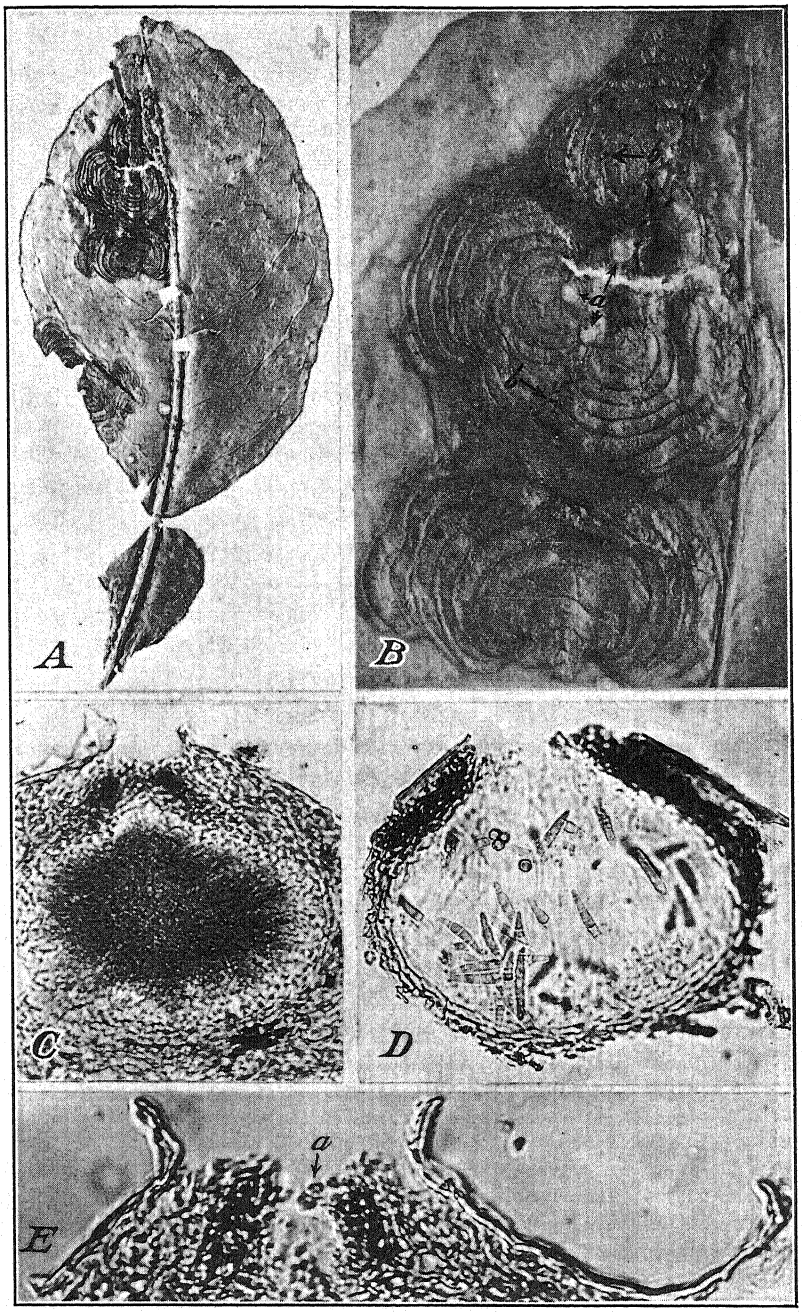


FIG. 1. A. Areolate spot of citrus on what is apparently sour orange, from Surinam, Dutch Guiana, collected by G. Stahel (x 1). B. Upper surface of large lesion shown in A (x 4); *a*, initial lesions reported by Bondar for the disease in Bahia; *b*, Coniothyrium pycnidia. C–E. Coniothyrium pycnidia from A, showing (C) colored conidial mass and (E, *a*) individual conidia in ostiole and (D) a perithecium of the Leptosphaeria from the specimen from Bondar referred to in the text (C × 325; D, × 380; E, × 500).

In attributing areolate spot to the Leptosphaeria, Bondar states that the disease is evidently not new. He cites an instance in which it was said to have been destructive in orange nurseries in Bahia 10 years earlier. Moreover, he considered that the same disease was reported in São Paulo as *Phyllosticta hesperidearum* Catt., as well as in Ceylon, as *P. disciformis* Penz. No citations are given, but careful search of the literature to which he may have had reference fails to disclose a distinctive disease corresponding to the description of areolate spot.

Bondar identified the Leptosphaeria as *L. citricola* Penz., which is described as occurring in Italy, but with no mention of the striking areolate spot of South American disease. In the writers' opinion areolate spot, clearly diagnosed by Bondar, is probably caused by a hitherto undescribed species of Leptosphaeria indigenous to South America, and as yet unknown elsewhere. Primarily, on the basis of Bondar's description and illustrations of the fungus, it is, therefore, here described under the name *L. bondari.*[4]—A. A. BITANCOURT, Instituto Biologico de São Paulo, Brazil, and ANNA E. JENKINS, Bureau of Plant Industry, United States Department of Agriculture, Washington, D. C.

Transfer of Diaporthe umbrina to the Genus Cryptosporella.—The fungus causing brown canker, one of the most important diseases of the rose in the eastern United States, was described as *Diaporthe umbrina* Jenkins[1] in 1918 and has since been so designated. Prior to the finding

[4] Technical description:

Leptosphaeria bondari, n. sp.

Asci 60–70 μ, in length, 8-spored, spores 3–5-septate, 21–24 μ long, becoming constricted at the septa, brown when mature; Coniothyrium pycnidia, spherical, somewhat flattened, 60–160 μ in diam., ostiole circular, surrounded by a darker color than the rest of the pycnidium, conidia 4 × 2–3 μ; similar pycnidia on the lesions, apparently also of this fungus, bear 4-septate conidia 20 μ in length; these were hyaline, although similar pycnidia from an isolation made from an ascospore of the Leptosphaeria yielded colored conidia.

Ascis 60–70 μ longis; sporis 3–5-cellulatis, 21–24 μ longis, demum ad septum constrictis; pycnidiis Coniothyrii 60–161 μ in diam.; conidiis 4 × 2–3 μ; conidiis forsan alius status conidici 4-septatis, 20 μ longis, hyalinis vel videtur demum coloratis.

Distribution: On leaves and twigs of *Citrus* sp., *C. aurantium* L., *C. grandis*, Osb., *C. sinensis* Osb. (Bahia Navel), also said by Bondar to occur on *C. limonia* Osb. and *C. medica* L., associated with and evidently producing the disease known as "areolate spot"; lesions reaching nearly 4 cm. in diam., initial spot about 1 mm. in diam., white and translucent, increasing progressively on one side, the dead tissue dividing into concentric ridges or zones, mature spots often pale at the center, and brown toward the outside, region surrounding zonation often conspicuously yellowed.

Type locality and type specimen: Brazil, Bahia, Bahia, on *Citrus sinensis* Osb., Nov. 1932, G. Bondar 337. In Herb. Phytopath., Inst. Biol. de São Paulo, Brazil, and in Mycol. Coll., Bur. Plant Indus., U. S. Dept. Agr., Washington, D. C.

Associated fungi: *Colletotrichum gloeosporioides* Penz., *Vermicularia* sp., and others.

[1] Jenkins, A. E. Brown canker of roses, caused by *Diaporthe umbrina*. Jour. Agr. Res. 15: 593–599. 1918.

of pseudoseptate spores, the very similar genus Cryptosporella was selected for its generic classification.[2] This genus had been chosen by Shear[3] for a similar fungus, also of somewhat indeterminate position and closely related to Diaporthe. Described as *C. viticola* Shear, this fungus possessed unicellular ascospores and a conidial stage typical of the genus Phomopsis, with both alpha and beta conidia. *Diaporthe umbrina,* on the other hand, produces conidia corresponding to the alpha type only in Phomopsis. In general these occur in irregular chambers within an ectostroma and may be more or less exposed at maturity. This *"Myxosporium"* type of fruit body is similar except for the shorter conidia, to the *"Fusicoccum"* type reported by von Höhnel[4] for several species of Cryptosporella.

The pseudoseptate spores in *Diaporthe umbrina* were observed in the spring of 1918, the second season the fungus was studied, in the National Rose Test Garden, Arlington Experiment Farm, Rosslyn, Virginia, where it was extremely abundant. The canker on which these spores were borne was apparently not of recent development and may have resulted from activity of the fungus during the preceding winter months. It was anticipated that ascospores showing more or less definite septation would be found frequently without making and special search for them. The fungus was, therefore, classified in the genus Diaporthe with explanation as to the absence of definite septation in the spores. But in the course of subsequent studies of the organism,[5,6,7] during which many specimens showing the perfect stage have been examined, unicellular spores have been the rule without exception.

It is likely that the genus Cryptosporella as now delimited includes several groupings, one of which would be the short, fusoid-spore type of Cryptospora and another the nonseptate-spore type of the rather heterogeneous genus Cryptodiaporthe. Inasmuch as certain species of Cryptodiaporthe are tardily septate, the general structure, occasional septation or near-septation, and the conidial stage of *Diaporthe umbrina* would suggest that this species would fall in this latter grouping. Under the circumstances, as has already been indicated,[8] *Diaporthe umbrina* is be-

[2] Unpublished data.

[3] Shear, C. L. The ascogenous form of the fungus causing dead-arm of the grape. Phytopath. 1: 116–119. 1911.

[4] Höhnel, F. von. Mycologische Fragmente. Ann. Mycol. 16: 106–109. 1918.

[5] Jenkins, A. E. Brown canker of the rose. Amer. Rose Ann. 1927: 161–182. 1927.

[6] ———. Development of brown canker of roses. Jour. Agr. Res. 42: 293–299. 1931.

[7] ———, and R. P. White. Identification of *Diaporthe umbrina* on rose from England. Mycologia 24: 485–488. 1932.

[8] Wehmeyer, L. E. The genus Diaporthe Nitschke and its segregates. 349 pp. Univ. of Michigan Press, Ann Arbor. 1933.

lieved to be of the genus Cryptosporella and is here transferred to that genus as **C. umbrina,** (Jenkins) n. comb.

A comparison of the Diaporthe on rose petals shown in figure 1, A,

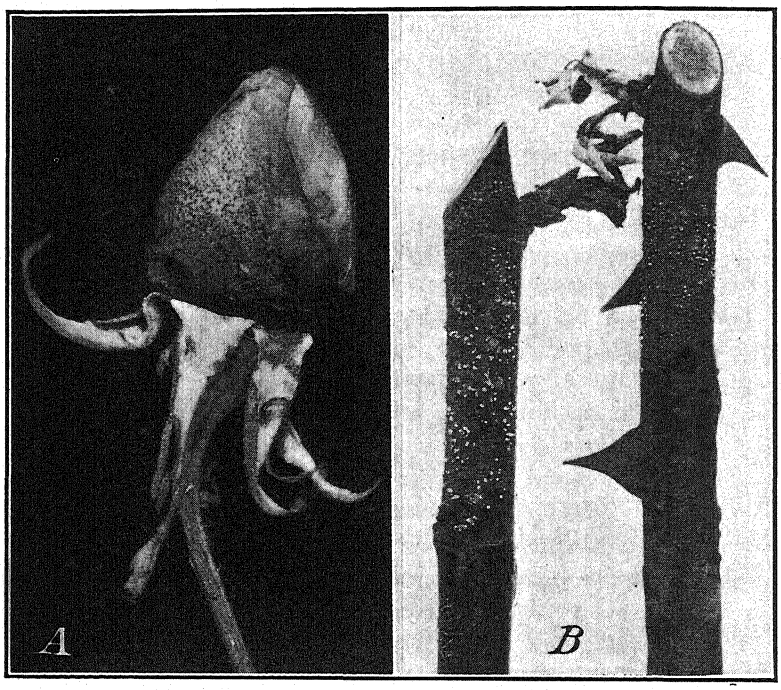


FIG. 1. Phomopsis on rose. A. On petals from Baton Rouge, La., May, 1924, C. W. Edgerton × approximately 1. B. On cuttings of *Rosa* sp. (var. La Guirlande) from a greenhouse, Arlington Experiment Farm, Rosslyn, Va., May, 1923, A. E. Jenkins. ×1.

with *Cryptosporella umbrina,* serves to illustrate the very close relationship between these 2 genera or species. The specimen represented was contributed by C. W. Edgerton in May, 1924, following the senior writer's request for material from Louisiana that might prove to be *C. umbrina.* Although the pycnidia on the petals were somewhat more prominent than is usual for corresponding fruit bodies of the Cryptosporella on this substrate, the alpha-type conidia were practically indistinguishable from conidia of the Cryptosporella. Only by a critical cultural study of the Diaporthe was it recognized as distinct from the Cryptosporella. In culture the Diaporthe grew somewhat more luxuriantly than does the Cryptosporella; moreover, both alpha and beta conidia were produced, and on corn-meal media in Erlenmeyer flasks the perfect stage developed. In

culture the latter stage in the case of the Cryptosporella has been observed only on rose-stem substrates. The asci and spores of the Diaporthe, however, were essentially indistinguishable from those of the Cryptosporella, *i.e.*, until the spores of the former became septate, which soon occurred.

The Diaporthe just discussed was previously reported without specific identification,[9] but it is here recognized as *D. eres* Nit., as broadly delimited by Wehmeyer in the monograph already cited. Several other specimens of Phomopsis from rose cankers from several different sources have been studied and cultured, more or less directly in connection with the investigation of *Cryptosporella umbrina,* but none of them has produced a perfect stage. One of these specimens is shown in figure 1, B. On the basis of their alpha and beta conidia, at least 2 of the strains are morphologically distinct from that of *D. eres* from Louisiana; nevertheless, it is probable that they all represent this same species complex.—ANNA E. JENKINS AND LEWIS E. WEHMEYER, Division of Mycology and Disease Survey, Bureau of Plant Industry, Washington, D. C., and Department of Botany, University of Michigan, Ann Arbor, Mich.

[9] U. S. Dept. Agr. Bur. Plant Indus. Plant Disease Rptr. Sup. 42, p. 360. 1925.

REPORT OF THE NINETEENTH ANNUAL MEETING OF THE PACIFIC DIVISION OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

The nineteenth annual meeting of the Pacific Division of The American Phytopathological Society was held at the University of California at Los Angeles, June 26–27, 1935, in conjunction with those of the Pacific Division of the American Association for the Advancement of Science and Affiliated Societies.

Four half-day sessions were held. At a short business meeting that preceded the presentation of papers, C. E. Owens of Oregon State College, Corvallis, Oregon, was elected President for the ensuing year. All other officers will continue to serve until 1936, when a general election will be held at Seattle, Washington, the next meeting place of the Society.

The attendance was not so large as anticipated, the average being only 30 members at each of the sessions. Forty people signed the register, the great majority of whom were members of the Society. Twenty-four papers were presented, the titles and abstracts of which accompany the present report.

Preceding the opening session of our society, the pathologists met with The Botanical Society of America, Pacific Section; The Society for Experimental Biology and Medicine, Pacific Coast and Southern California Branches, and The Western Society of Naturalists, at a joint symposium on "Virus Diseases of Plants and Animals," which was both eminently successful and instructive. C. W. Bennett of the U. S. D. A., Riverside, was presented by the pathologists as their speaker, and his paper entitled, "The Relation of Viruses to Plant Tissues," was a distinct credit to him and the Society that he so ably represented. Dr. Bennett did not make the mistake of attempting to cover the entire subject of virus diseases as they affect plants and thereby run the danger of presenting a paper too sketchy to be of value, but rather confined himself sharply to a single phase of it—a phase in which so much greatly diversified and controversial opinion exists. His digest of the available evidence bearing on his subject was not only complete but arranged to show the fields in which experimental data are still lacking or insufficient. He clarified certain existing misconceptions of the relationship between viruses and certain plant tissues, and drew deductions from existing evidence that were at once properly conservative and logical. Lastly, he offered many meaty suggestions to future workers in this very important field of plant diseases.

Following the symposium an hour was devoted to a demonstration of techniques applicable to virus-disease investigations by various members of

the societies that had participated in the symposium. The pathologists were well represented at this session by Messrs. Carsner, Thornberry, and Takahashi, who gave practical and interesting demonstrations of various methods of plant inoculation, the preparation of virus filters, the use of stream double refractions, in the study of virus particles as well as other techniques.

B. A. RUDOLPH, *Secretary-Treasurer*

Experiments on the Multiplication of Curly-Top Virus in the Beet Leaf Hopper.—J. H. FREITAG AND H. H. P. SEVERIN.

The Effect of Carbon Dioxide on the pH Gradient of the Sugar Beet and on the Feeding of the Curly-Top Vector.—J. M. FIFE AND V. L. FRAMPTON.

The Effect of Clean Fallow on Field Populations of the Root-Knot Nematode Heterodera marioni.—J. M. OLIVEIRA.

California Celery Mosaic Diseases.—HENRY H. P. SEVERIN AND JULIUS H. FREITAG.

Two celery mosaic diseases with different symptoms and host ranges occur in California. Common names proposed for these diseases are western celery mosaic and celery calico.

The symptoms of western celery mosaic are similar to those of southern celery mosaic (Celery virus 1) but the viruses differ in host ranges. Celery calico is readily distinguished in the field by the green and amber mottling on the outer leaves, rarely on the younger leaves.

Ten species of aphids are vectors of western celery mosaic and celery calico; five of these species are known to breed on celery under natural conditions. The melon aphid, *Aphis gossypii*, transmits the 2 celery-mosaic viruses by contamination of mouth parts; each virus was transmitted by single, previously noninfective, melon aphids after feeding 5 minutes on diseased plants and then 5 minutes on healthy plants. An undetermined species of aphid belonging to the genus Aphis, tested singly, transmitted the virus of western celery mosaic by internal mechanical transmission in from 4 to 7 hours but not daily to healthy plants when transferred. Large populations of different species of aphids reared on western-celery-mosaic plants and transferred daily to healthy celery plants transmitted the virus during the first day but not on the following days.

A New Blight of Pea in California.—WILLIAM C. SNYDER.

Following a wet spring and other conditions favoring infection and spread of certain foliage diseases, severe blighting of the leaves, stems, and pods of pea, *Pisum sativum*, was observed in several market-pea areas of California early in the 1935 season. Although the principal causes of blight were found by isolation, inoculation, and taxonomic studies to be *Mycosphaerella pinodes* (Berk. and Blox.) Stone, *Ascochyta pinodella* Jones, and *A. pisi* Lib., an additional and apparently undescribed fungus differing both macroscopically and microscopically from the foregoing, was isolated and proved in inoculation trials with monosporal cultures to be actively parasitic. The new disease occurs on the leaves, stems, and pods of the common market varieties so far observed, with symptoms somewhat resembling those caused by *M. pinodes*. In pure culture the fungus produces large, black pycnidia abundantly, but on oat agar has not been found to yield the conidial exudate generally produced by the known blight organisms. Pycnidial exudate from

stems is composed of predominately nonseptate, nonconstricted, hyaline conidia that approximate or exceed in size pycnospores of *A. pisi* or *M. pinodes*. Septate conidia have been found infrequently, even at the time of their germination.

An Experiment to Determine the Susceptibility of Flax to Verticilliosis.—B. A. RUDOLPH.

The production of flaxseed and linseed oil is an industry comparatively new to California and is increasing rapidly at several points in the State. Recently the question arose as to the suitability of flax as a rotation crop to follow cotton on those soils where verticilliosis has become a problem. Apparently there is no published report of the disease in flax. It has never been recognized in flax fields in California. At the University of California Deciduous Fruit Station at San Jose, pots of steam-sterilized soil were inoculated with varying quantities of the pseudo-sclerotia of *Verticillium albo-atrum* R. & B., originally isolated from cotton and produced by the fungus on Czapeck's agar. Disinfected flaxseed was planted in these pots. Suitable checks were provided. The plants grown in pots of inoculated soil were sickly and remained stunted. The bolls matured earlier and were smaller than those of healthy plants. The leaves of the diseased plants also were smaller and turned yellow and died early. The symptoms of the sick plants were generally those ordinarily presented by other plants affected with verticilliosis. No internal discoloration of the main stems was noted, however, which may or may not have been occasioned by the conditions under which the experiment was conducted. The fungus was isolated from the main stem of a number of diseased plants an inch or more above ground. This preliminary experiment indicates that flax (Punjab) might, if planted in soils in which *Verticillium albo-atrum* has become established through the medium of cotton, contract verticilliosis.

To Visualize a Distinction between Viruses and Organisms.—J. LEE HEWITT.

Particles in a fluid medium are in motion. We may, by way of hypothesis, say that rhythmic motion of the ultimate particles of living matter is the determinant of life. If a particle of material should be of such structure, with such a distribution of masses and such a security of chemical linkage that it could be set into rhythmic motion and maintain its identity, the rhythmic motion would tend to persist at an optimum of temperature and other environmental factors. The particle would tend to grow by addition of other similar material and would reach a limit and divide. It would tend to encourage synthesis of the constituent parts by whatever agency they were being built. It would respond to a stimulus by orientation or motion or growth.

From this beginning up to the realm of microorganisms there is quite room enough for the existence of whole categories of living material, including ultramicroscopic nucleate organisms, simpler organisms and unorganized virus.

A virus might arise *de novo* in one of three ways, by attack of a pathogen on a living cell element, by incidence of a chemical material upon the element, or by stress of marginal conditions for life.

Viruses or very simple organisms might undergo organic change, such as attenuation.

Aeroplane Dusting With Sulphur to Combat Stem Rust of Wheat.—W. W. MACKIE.

In 1935 an epiphytotic of stem rust in the San Joaquin Valley, California, was combatted by aeroplane dusting with sulphur. Approximately 25 pounds per acre were applied. All of the sulphur used passed through a 325-mesh screen and was included in 4 brands. From April 29 to May 14, 22,000 acres were dusted. The dusting operations began at daylight and continued until about 9 a. m., when convection currents of warm surface air caused the sulphur to rise in clouds. As many as 13 planes were in the field at the same time. The dust was most effectively applied between anthesis

and hard-dough stages, a period of approximately 21 days. Although 3 dustings were recommended, most of the fields received but one. One application halted the rust attacks about 7 days, when rust attacks began again and continued until checked by unfavorable weather or by the maturity of the wheat. Where the second dusting was applied 10 days after the first, very satisfactory control was secured. Uredia forming just before the second application were destroyed or halted before sporulation occurred.

An Unidentified Species of Sphaeropsis on Maize in California.—W. W. MACKIE, HELEN JOHANN, AND N. E. STEVENS

In the autumn of 1933 diseased stalks of maize were found in the plots at Berkeley. During the following year the disease appeared to increase considerably. A few isolated specimens were found in corn fields of the delta region, near Stockton. The diseased plants matured somewhat prematurely, bearing stunted ears with shriveled kernels. Numerous lesions were borne on the first, second, or third internodes only, and are in appearance much like uredia of stem rust. These lesions erupt while the ears are maturing. The prematurely ripened stalks become brittle and readily break under wind or other stresses. Helen Johann, of the Division of Cereal Crops and Diseases, United States Department of Agriculture, placed the fungus in the genus Sphaeropsis. On media consisting of agar and cornstalk shavings the mycelial growth was light, but pycnidia were produced bearing unicellular pycniospores. On oat agar a heavy grayish green stroma developed but no pycnidia appeared. The spores probably averaged 15 μ in length, which is short for the common *Sphaeropsis malorum* but is not greatly different from *S. ambigua* (Saccardo 3: 304), the lone species so far discovered reported on corn. An examination of old cornstalks that had over-wintered at Berkeley disclosed only insect-mutilated pycnidia from which the perfect form could not be identified. Further identification will be necessary before this species of Sphaeropsis can be definitely placed.

Observations on a Species of Ligniera, a Root Parasite of Stellaria media.—J. T. BARRETT AND HORACE PARKER

While making a survey of chytridiaceous parasites of root and root hairs of higher plants a species of Ligniera was found abundantly and in all stages of development in *Stellaria media.* A comparison with the published descriptions of *L. junci* (Schwartz) Cook suggests that our form is that species. A careful study of living material suggests that the life cycle starts by infection of the plant by swarm cells originating from resting spores. The resulting myxamoeba give rise to zoosporangia that produce a few (1–6) biciliate zoospores. These develop rapidly, retaining a more or less spherical shape at first, and give rise to the groups of resting spores. The cilia, one long and one short, are retained on the growing zoospore within the host cell often until the zoospore has quadrupled in size. Germination of the zoosporangia and penetration of the host wall have been observed but not the germination of the resting spores. Empty spore cases have frequently been seen in partially disintegrated roots.

A Phytophthora Root Rot of Cauliflower.—C. M. TOMPKINS, C. M. TUCKER, AND M. W. GARDNER

A root rot of cauliflower, *Brassica oleracea* L. var. *botrytis* L., caused by *Phytophthora megasperma* Drechsler, occurs in winter plantings in the coastal areas of central California. Low, poorly drained areas and low temperatures are the principal predisposing factors. Characteristic symptoms consist of reddening or purpling of the leaves, sudden wilting, and invasion and decay of the root system. Plants of all ages are susceptible. Cauliflower seedlings (6 to 8 leaves), in 6-inch pots of autoclaved soil in

buckets containing 4 inches of water were inoculated with pure cultures of the fungus, and placed outdoors at 14° to 20° C. The incubation period is approximately 3 weeks. Healthy cauliflower seedlings were subsequently inoculated and infected with the reisolated fungus. The growth optimum of the fungus on malt extract agar is 19° to 22° C. Brussels sprouts, *B. oleracea* var. *gemmifera* Zenker; cabbage, *B. oleracea* L. var. *capitata* L.; and stock, *Mathiola incana* R. Br., are subject to natural infection, while stock and wallflower, *Cheiranthus cheiri* L., were artificially infected. The fungus produces oogonia and oospores resembling those of *P. megasperma*, but slightly smaller; both amphigynous and paragynous antheridia are present, the latter in much greater numbers. Sporangia, which are rarely found, are nonpapillate. In cultural characters and temperature relations the fungus behaves identically with *P. megasperma*. The differences in sizes of oogonia and oospores are regarded as too small to warrant the separation of the cauliflower fungus, especially in view of the very close correspondence in other characters.

Crown Gall on Conifers.—C. O. SMITH

Conifers have only recently been listed as attacked by the crown gall organisms, *Pseudomonas tumefaciens* Smith and Townsend. The literature records natural crown galls on *Cupressus arizonica* Green and *Juniperus sabina* Linn. W. T. Horne, Plant Pathologist, University of California, in conversation with the author, reported observing in 1910 a lawn tree of *Libocedrus decurrens* that had a typical crown gall 3 to 4 inches in diameter. In 1916 the author studied a gall on nursery trees of *Libocedrus decurrens*, isolated the causal organism, *Ps. tumefaciens*, and produced, with these cultures, galls on *Salix* sp., on *Prunus cerasifera*, on *Cupressus sempervirens*, and on tomato. Galls from puncture inoculation with *Pseudomonas tumefaciens* isolated from peach have been produced during 1934 and 1935 on the following species of conifers: *Cupressus arizonica*, *C. arizonica* var. *bcnita*, *C. lusitanica*, *C. guadalupensis*, *C. macrocarpa*, *C. sempervirens*, *Chamaecyparis lawsoniana*, *Juniperus procera*, *J. cedrus*, *J. hierbernica*, *Thuja orientalis*, *T. plicata*, *T. occidentalis*, *Sequoia sempervirens*, *S. gigantea*, *Pseudotsuga taxifolia*, and *Libocedrus decurrens*. Crown gall is produced artificially on conifers with great difficulty. The inoculation wounds heal over with some swelling of tissue around the injury, and the results at first appear to be doubtful or negative, but some months later several knob-like enlargements may develop from the healed tissue. These are small (1 to 2 millimeters), but they continue to increase in size and may coalesce. On *Sequoia gigantea* galls more typical in size and shape have developed.

An Outbreak of Curly Top on Pansy.—B. F. DANA AND F. P. MCWHORTER

An important pansy-seed industry in Oregon was menaced in 1934 by a serious outbreak of curly top. On July 6, 20 per cent of the plants in a 5-acre field showed severe injury. Mild injury appeared on many other plants. No difference in susceptibility could be detected in named varieties or variety mixtures, excepting a blue-flower foreign strain with 80 per cent severely injured plants. Dwarfing and rosetting of shoots were prominent symptoms. Flower size and seed production were strikingly reduced. Diseased plants ceased blooming early and gradually deteriorated, many dying before the end of the season. Adults and nymphs of the beet leaf hopper, *Eutettix tenellus*, were abundant on all plants examined July 6. The severely diseased plants and the large numbers of nymphs indicated that the pansy was a favorable host for the leaf hopper and also for the curly-top virus. Plants grown from seed produced by diseased plants were disease-free, thus indicating lack of seed transmission of the curly-top virus. This also was in line with experience of the grower in perpetuating breeding stock.

Root rot of aster caused by Phytophthora cryptogea.—C. M. TOMPKINS, C. M. TUCKER, AND A. E. CLARKE

Root and stem rots caused by *Phytophthora cryptogea* have been reported on *Aster* sp. in England and Ireland and on China aster, *Callistephus chinensis*, in Australia. In August, 1934, a root disease of China aster, caused by *P. crytogea*, was observed in Golden Gate Park, San Francisco, and later in aster plantings in other coastal areas. Low, poorly drained soils favor infection. Fungus invasion occurs at or near the base of the tap-root and progresses upward to the base of the petioles, producing a blackish brown discoloration of the tissues. Root invasion causes a sudden loss of turgidity in the leaves with complete collapse of aerial parts, followed by early death of the plant. Young, healthy China asters, variety Late Branching Mary Semple, grown in 6-inch pots of moist, autoclaved soil, were successfully inoculated in the greenhouse at 18° C. with pure cultures of *P. cryptogea*. Aster plants of the same variety were later inoculated with the reisolated fungus and infection obtained. In greenhouse tests complete susceptibility to infection was shown by 45 varieties of China aster in 10 classes, including Fusarium wilt-resistant types. On oatmeal agar the fungus produces abundant white cottony mycelium with occasional clusters of small, hyaline, thin-wall vesicles, but no sporangia or oogonia. Hyphae transferred to Petri's solution or a nonsterile soil suspension develop long-ovate to obpyriform, nonpapillate sporangia averaging about 33 x 17.5 microns, with a small, refringent, thickened region at the apex. Ooogonia were not observed. Optimum growth occurs at 25° to 28° C. and ceases at 35° C. In morphologic, physiologic and pathogenic characters the fungus agrees closely with *P. cryptogea*. This is believed to be the first definite record of the occurrence of the species in America.

Observations on the Pathological Histology and Phyto-chemistry of Psyllid Yellows.—J. R. EYER.

The manner of entrance of the feeding stylets of the potato psyllid nymph into the vascular tissues of the leaf is described. The destruction of border parenchyma cells at the feeding objective and the deposits of starch in the surrounding mesophyll of the leaf and the cortex and pith of the stem are characteristic of the disease. No x-bodies were observed in diseased foliage, nor was any necrosis of the phloem of the stem apparent. The chloroplasts revealed disintegration, especially near the feeding punctures and generally throughout leaf tissues showing marked disease symptoms. The sheath materials surrounding the stylets are apparently of animal origin, and contain chitosan and musin. Certain plant tissue disintegration products also were demonstrated in the surrounding tissues. These contained arginine, tryptophane, and possibly tyrosine. Quantitative estimations of the carbohydrates in healthy and diseased plants confirmed the abnormally high starch values for diseased leaves and stems, although it was further observed that these decline rapidly after the plant reaches maturity. The definite positive relationship between hexoses and starch present in the healthy plant is abrogated by the disease. Sucrose, the principal sugar of translocation, was found to be absent or its formation inhibited in the vegetative portions of diseased plants.

Toxicity of Compounds of Ammonia to Sclerotium rolfsii.—A. E. DAVEY AND L. D. LEACH

In field trials, reported elsewhere, it was found that in soils of pH 7.0 and pH 7.9 ammonium sulphate was as effective as anhydrous ammonia in reducing loss to sugar beets from southern sclerotium rot. In an attempt to explain the action of ammonium sulphate, laboratory tests were made of the effect of ammonium sulphate in solutions buffered with calcium carbonate, solutions of ammonium carbonate (bicarbonate) and ammonium phosphates.

It was found that solutions of ammonium sulphate containing 82.4 millimols of

ammonia (NH_3) per liter (pH 6.0) and solutions of monammonium phosphate with the same ammonia content (pH 4.5) did not injure mycelium of *Sclerotium rolfsii* immersed in them. Solutions containing 41.2 millimols of ammonia as ammonium sulphate and saturated with calcium carbonate giving a pH of 7.8 were toxic to mycelium within 5 days. Solutions of ammonium bicarbonate and dibasic ammonium phosphate with a pH of 7.5 and concentrations respectively of 17.8 and 14.7 millimols of ammonia were also lethal, within 5 days, to immersed mycelium.

The above results suggest that ammonia, applied to the soil as solutions of anhydrous ammonia or ammonium salts, may, with a sufficiently high pH, be the effective agent in reducing the extent of infection by inhibiting the growth of the fungus.

Soil Amendments for Southern Sclerotium Rot of Sugar Beets.—L. D. LEACH AND A. E. DAVEY

The percentage of infection of *Sclerotium rolfsii* on sugar beets was reduced from 60 per cent in untreated plots to 25 per cent in plots treated with cyanamid at the rate of 1000 pounds per acre applied 3 weeks prior to planting. In the same experiment, plots treated with 10 tons of manure per acre showed 40 per cent infection. Hydrated lime at 5 tons per acre or ammonium sulphate, side-dressed at 1000 pounds per acre, did not significantly reduce the percentage of infection. The yields in tons per acre of disease-free sugar beets from the plots were as follows: Untreated, 7.8; cyanamid, 16.8; manure, 11.1; hydrated lime, 10.9 and ammonium sulphate, 10.8.

In another series of plots, application of either anhydrous ammonia of ammonium sulphate in irrigation water at the rate of 200 pounds of nitrogen per acre decreased the percentage of infected beets from 30 to 9 per cent and increased the yield of disease-free beets from 11.5 to 19.7 tons per acre.

Applications of anhydrous ammonia in irrigation water to sugar beets, after 6 per cent of the plants were killed by the fungus, reduced the final percentage of infection but did not provide a practical control.

The Relation of Penicillium vermoeseni to a Disease of Ornamental Palms.—DONALD E. BLISS

A leaf-base rot of *Phoenix canariensis*, a bud rot of *Washingtonia filifera*, and a trunk canker of *Cocos plumosa* are responsible for the loss of many ornamental palms each year along the Pacific Coast in southern California. Although differing in outward manifestations, these diseases are similar as to cause. *Penicillium vermoeseni* Biourge (*P. roseum* group) was isolated from the affected tissues of the 3 palm species. Controlled inoculation and reisolation experiments with these isolates, together with a similar culture from *Phœnix dactylifera*, proved the pathogenicity of each isolate on its respective host. Cross inoculations using cultures from *Ph. canariensis* on *W. filifera* and *C. plumosa* indicated that the various cultures of *P. vermoeseni* were alike pathogenically and about equally virulent. Inoculations were made through wounds, except on *W. filifera* in which moldy bran was shaken between young, folded pinnae.

Dissection of diseased palms showed that in *Phoenix canariensis* death was caused by the successive decay of leaf bases from the oldest to the youngest; that in *W. filifera* it was due to mycelial invasion of the young, tightly folded leaves in the bud; and that in *C. plumosa* it was caused by the weakening and breaking of the trunk.

The Symptoms of Narcissus Mosaic Developed Within the Plant.—FRANK P. MCWHORTER

Narcissus mosaic leaf symptoms, evident externally, result from demonstrable cell and tissue changes within the epidermal and outer chlorenchymatous regions. The squarish mottle or mosaic pattern is in part delineated by the cross-veining that forms

a right-angle network within the narcissus leaf. Roughening of the leaf and stem surface is a true and important symptom of narcissus mosaic. In all positive mosaic cases investigated cytologically, hypertrophy of epidermal cells or of chlorenchymatous cells just beneath the epidermis, is a constant symptom of diagnostic value. X-body formation occurs in some varieties of mosaic-diseased narcissus but is of little diagnostic value. Initial hyperplastic activity within chlorenchyma subtended beneath the affected epidermis produces epidermal galls. The cells composing these exhibit coarse vacuolation and are bounded by walls broken down into component striae. Cuticular membranes are disrupted. These cell changes exhibit facilities for excess water loss and partly explain the tendency to wilt characteristic of mosaic individuals.

A Peach Tree Disease Recently Discovered in California.—H. S. REED AND H. H. THORNBERRY

The disease came into prominence in one of the important peach districts of southern California in the spring of 1933 and has increased in severity and distribution each succeeding year. Since the nature of the disease has not been determined, we have temporarily designated it as the "1933 peach tree disease." Trees affected by it are not killed but assume an extremely unthrifty condition. Fruit production is reduced to a point where the orchards do not pay expenses. The leaves of affected portions of trees are small and usually show yellowing or irregular areas that occasionally drop out, forming numerous holes. Severely affected leaves fall from the trees by the end of June and are succeeded by apparently healthy ones. The first foliage, however, produced the following spring, again shows typical symptoms, and the tree is less thrifty than in the preceding year. Similar symptoms have been found on almond, plum, and prune foliage. Experiments have been started to determine the transmissibility of the disease, and observations are in progress to learn something about the susceptibility of different varieties.

Endoxerosis of Lemon Fruits as Affected by the Application of Different Amounts of Irrigation Water.—E. T. BARTHOLOMEW

Lemon trees were grown in large lysimeter tanks. The soil had a moisture equivalent of 11 per cent and a wilting point of 4 per cent. Water was applied to one group of these trees when the soil had reached a moisture content of 6 to 8 per cent; to the second group when it had reached 5 to 6 per cent; and to the third group when it had reached 3½ to 5 per cent. The yields of endoxerotic fruits, arranged in descending order, were third, first, and second groups, respectively. On the whole, the results of these tests indicate that, while a high soil moisture content is conducive to the production of endoxerotic fruits, yet, when the period of stress (hot weather) is great and is sufficiently extended, those trees having access to the least amount of water will show the greatest yield of endoxerotic fruit.

The Interpretation of Oregon Tip Blight on a Basis of Causal Viruses.—FRANK P. MCWHORTER AND J. A. MILBRATH

During the last 4 years (1930–1935) a tomato disease, locally called tip blight, has been of great economic importance in the Southern Oregon canning areas. Typical diseased plants exhibit severe blighting of the terminal shoot as the most noticeable symptom. Small plants are killed; larger (older) plants soon lose their ability to produce marketable fruit. The general condition suggests "streak" but this interpretation has been rendered untenable both by symptom study and by test inoculations. Inoculations by grafting and by carborundum methods relate the Oregon trouble to

two viruses: "Spotted Wilt" and an undetermined virus. The undetermined one produced (1934) most of the tip-blight cases, is very difficult to transfer from tomato to tomato, and has thus far failed to produce symptoms on any other solanaceous host. On tomato it is a quick-killing virus, selectively active on terminal shoots, and first evidenced by arrested growth and black, usually large, necrotic spots on leaves.

The Properties and Interpretation of Tulip-Breaking Viruses.—FRANK P. MCWHORTER.

The theory that tulip breaking results from the interaction of two antithetic viruses one of which tends to add flower color and one to remove flower color, has been substantiated by actual synthesis of normal breaks by inoculating unbroken tulips with mixtures of viruses. The color-removing virus is dominant. A juice inoculum consisting of 10 parts of color added to 1 part of color-removing, resulted in the synthesis within a group of inoculates of a large proportion of normal breaks comparable to commercial Rembrandts.

We have been unable to distinguish or separate the 2 viruses on a physical-property basis. Some of the properties are: Thermal death point lies between 65° and 70° C.; infectious in dilutions up to 1/100,000; are not destroyed by drying (leaves) for 11 days; resistant to alcohol; easily transmitted by hypodermic injections; not transmitted by gentle rubbing; inoculations to onion, tomato, iris, narcissus, petunia, Tropaeolum, Brodea and Camassia were negative. The tulip viruses are very closely related to those frequently present within the bulb-perpetuated lilies.

An Explanation of Rhiomyxa hypogaea Borzi.—J. T. BARRETT

While making observations on a species of Ligniera in the roots of *Stellaria media*, oospores of a fungus were found that closely resembled those illustrated and described by Borzi as a part of the life cycle of his *Rhizomyxa hypogaea*. The oospores often are associated with the Ligniera, but may occur in roots in the absence of the latter fungus. Associated with this sexual stage, which was observed in all stages of development, are large oval to long zoosporangia that discharge biciliate swarm spores through very thick exit tubes. No vesicle has been definitely observed. As seen in roots of *Stellaria media*, the zoosporangia occur singly. Very recently the same sexual stage was observed in the roots of sugar beet associated with similar zoosporangia joined in a chain of 3 to 6. In one case all zoosporangia discharged zoospores almost simultaneously. The studies thus far made suggest that Borzi's *Rhizomyxa hypogaea*, as described, may have been an association of Ligniera, perhaps *L. junci* (Schwartz) Cook, and an undescribed species of the order Ancylistales.

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CHEMICAL STUDIES ON THE VIRUS OF TOBACCO MOSAIC

IV. SOME EFFECTS OF DIFFERENT CHEMICAL AGENTS ON INFECTIVITY[1]

W. M. STANLEY

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INTRODUCTION

The effect of various chemical agents on the infectivity of tobacco-mosaic virus has been studied by many workers. The earlier investigators, including Koning (18), Heintzel (10), Allard (1, 2), and Chapman (4), appear to have been interested in obtaining an insight into the nature of viruses by studying the effect of various chemicals on infectivity. The most extensive experiments were those of Allard, who found that, with the exception of acids, bases, potassium permanganate, zinc chloride, copper sulphate and antiformin in concentrations of 1 per cent or greater, all of the many chemicals which he tested left the virus preparations infectious. He also found that the virus was adsorbed on aluminum hydroxide. Somewhat later Duggar and Armstrong (6) found that pokeweed juice had an inhibiting effect on the infectivity of tobacco-mosaic virus. McKinney (21) reported similar results with juice from cucumber plants. Vinson and Petre (33) found that suspended matter tended to cause an increase in the infectivity of tobacco-mosaic virus. Johnson (15) reported that bubbling air or oxygen through preparations for several weeks attenuated tobacco-mosaic virus. Johnson (14, 16) and Johnson and Grant (17) studied the effect of ethyl alcohol, nitric acid, and formaldehyde on several viruses and suggested that reaction to chemicals might be used as an aid in classification. Johnson also pointed out the possibility of separating mixtures of viruses by the use of chemicals having a toxic or inactivating action on one of the components. Fukushi (8) found that tobacco-mosaic virus was very resistant to many agents such as aconitine, morphine, strychnine, saponin, and digitalin, and was rendered noninfectious by oil of mustard, nicotine, and atropine. Fukushi (9) also reported that tobacco-mosaic virus was readily adsorbed on kaolin, aluminia, and aluminum hydroxide gel. Henderson (11) re-

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ported that phenol reduced the rate of inactivation of tobacco-ring-spot virus and Bald and Samuel (3) found that sodium sulphite retarded the inactivation of tomato-spotted-wilt virus. Burt Johnson (13) reported that, of a number of salts tested, only ammonium sulphate was useful in salting tobacco-mosaic virus out of solution. Shapovalov (26) recently reported that cobalt, nickel, and zinc sulphates, at certain concentrations, appeared to be somewhat toxic to both component viruses of tomato "combination streak," so that occasionally one or the other survived, thus allowing a separation. Mulvania (22), Purdy (24), Matsumoto (20), Silberschmidt (27), and Chester (5) found that both normal and immune serum of rabbits reduced the infectivity of tobacco-mosaic virus, and Chester reported that, in the case of normal serum and certain other proteins, the reduction was due to a decrease in the susceptibility of the host.

Studies on the effect of chemicals on viruses have been directed toward 3 objectives: firstly, as an aid in gaining an insight into the nature of viruses; secondly, as an aid in the classification of viruses; and thirdly, as an aid in the separation of mixtures of viruses. In practically all cases, experiments have been made by adding chemicals to virus preparations and then determining the infectivity of the resulting mixtures at once and after given periods of time. It has been the general practice to assume that virus is inactivated whenever the mixtures fail to prove infectious. However, it has been shown recently (28) that the loss of infectivity, which occurs when trypsin or certain other proteins are added to tobacco-mosaic and other viruses, is due to inhibition of infectivity and not to inactivation of virus, inasmuch as on the removal of such proteins the virus again manifests its infectivity. As was pointed out, it seems likely that at least part of this inhibition is due to some effect on the cells of the test plant used to measure the infectivity of the mixture. It is possible that other chemicals may have a similar inhibitory action, and hence that many chemicals which, in the past, have been regarded as possessing a toxic or inactivating influence on virus, may be, in fact, only inhibitory agents. This could, of course, easily lead to erroneous conclusions regarding the nature of viruses, and to erroneous views regarding their relationships. It seemed desirable, therefore, to determine whether or not loss of infectivity on addition of different chemicals is due to inactivation of virus or to an inhibitory action. It was also desirable to gain some idea concerning chemicals having little or no effect on virus, which might be useful in connection with work on concentration and purification of virus. Furthermore, it appeared likely that a study of virus in the presence of different chemicals would yield information concerning the general nature of the virus of tobacco-mosaic, and indicate whether or not virus acts as a protein. The present paper records the results of tests with 110 different chemicals.

MATERIALS AND METHODS

All chemicals used in the experiments to be reported were of reagent quality. Samples of purified virus, prepared by a modification of the method of Vinson and Petre (33) from juice of mosaic-diseased plants of *Nicotiana tabacum* L. variety Turkish were used in all experiments, unless otherwise specified. The purified virus preparations were diluted with 0.1 M phosphate buffer at pH 6.8 to 1:10 or 1:100 in order to facilitate counting by reducing the average number of lesions produced. To 10 cc. or larger portions of diluted purified virus was added, with vigorous stirring, 1 per cent by weight, unless otherwise specified, of the chemicals to be tested. These mixtures were then immediately tested against a control for virus infectivity by the half-leaf method previously described (12, 25, 28). These tests for virus infectivity were made by comparing the average number of lesions per half-leaf obtained on 5 or more half-leaves of *Nicotiana glutinosa* L. or 12 or more half-leaves of *Phaseolus vulgaris* L. variety Early Golden Cluster on inoculation with the preparations containing various chemicals, with the average number of lesions obtained on the other halves of the same leaves on inoculation with the control preparation containing no chemical. After standing 3 days at 5° C., the preparations were again tested for virus infectivity. Whenever mixtures containing chemicals produced about the same number of lesions as the control at once and after 3 days, it was assumed that the chemicals had affected neither the virus nor the plant used in measuring infectivity. When the mixtures produced about the same number of lesions as the control at once and a decreased number after 3 days, evidence was brought that the chemical did not affect the plant but that it slowly inactivated the virus. However, when mixtures produced no lesions, or produced a decreased number of lesions as compared with the control at once and after 3 days, it is impossible to determine whether the reduction was due to inactivation of virus by the chemicals or to an inhibitory effect on the plant which prevented the virus from manifesting its infectivity. In the majority of such cases, however, it was found possible to differentiate between these two possibilities by a method involving the removal of chemicals from the mixtures by dialysis. The preparations from which chemicals had been removed by dialysis were tested for virus infectivity against dialyzed controls. Any change in infectivity was assumed to have been caused by the action of the chemical on the virus. The samples to be dialyzed were placed in 4- to 24-inch lengths of ¾-inch transparent seamless Du Pont cellophane tubing containing a small glass marble and tied at either end with linen thread. The dialyses were carried out using a Kunitz and Simms (19) rocking apparatus and dialyzing against running distilled water for 4 hours, or until tests indicated that the chemical had been removed.

EXPERIMENTAL

Action of Different Chemicals on Tobacco-mosaic Virus

Table 1 records the results obtained with chemicals which were found to have little or no effect on the infectivity of the virus when tested in the presence of the reagents immediately after their addition and after standing for 3 days at 5° C. The fact that there was but slight change in the number of lesions is an indication that this group of 41 different chemicals has no appreciable effect on the virus or on the *Nicotiana glutinosa* plants used to measure its infectivity. Plants of *Phaseolus vulgaris* appeared to be affected by some of the reagents, especially those causing an acid reaction, such as citric acid, phthalic acid, and phenol. It has already been demonstrated (29) that an increase in the acidity of virus preparations beyond about pH 5 causes a marked reduction in the number of lesions produced on *P. vulgaris,* and but only a slight change in the number of lesions produced on *N. glutinosa.* For this reason the results obtained with *N. glutinosa* are regarded as the more significant. It may be seen from table 1 that, in general, the numbers of lesions produced by the chemical-containing virus preparations are about the same as the numbers produced by the control preparation.

Results obtained with a few chemicals that were found to cause an increase in virus infectivity are given in table 2. Certain of these reagents, such as dipotassium phosphate, sodium thiosulphate, phenolphthalein, and pyridine, cause a decrease in the hydrogen-ion concentration. This is probably the cause of the increased infectivity, since it has been demonstrated (29) that a decrease in the hydrogen-ion concentration from about pH 7 to pH 8 or 9 causes an increase in virus infectivity. Other of the reagents, such as benzidine, l-menthol, and sulphur, which are relatively insoluble, probably cause an increase in infectivity through their action as suspended particles.

The results obtained with a group of some 40 different reagents which were found to cause a decrease in the infectivity of virus are given in table 3. The hydrogen-ion concentration of the mixture of chemical and virus solution is given in most instances. It may be seen that, with perhaps one exception, the chemicals are either oxidizing agents, protein-precipitating agents, or reagents causing a hydrogen-ion concentration injurious to virus, test plant, or both. It is known (29) that reactions greater than about pH 10 and less than about pH 3 cause inactivation of virus, hence the hydrogen-ion concentration caused by reagents such as bromine, diisoamylamine, ferric chloride, oxalic acid, picric acid, stannic chloride, and platinic chloride would alone be sufficient to account for the inactivation. The oxidizing agents, including hydrogen peroxide, chloramine-T, potassium

TABLE 1.—*Infectivity tests on purified virus preparations immediately after the addition of various chemicals and after standing for 3 days at 5° C. Reagents in this group were found to have but slight effect on the infectivity of the virus*

Chemicals tested	Tested immediately after mixing				Tested after 3 days at 5° C.			
	Phaseolus vulgaris		Nicotiana glutinosa		Phaseolus vulgaris		Nicotiana glutinosa	
	Chemical	Chemical ÷ control	Chemical	Chemical ÷ control	Chemical	Chemical ÷ control	Chemical	Chemical ÷ control
1% aniline	33.2[a]	60.0[b]	101.2[a]	103.0[b]	13.2	99.0	72.5	103.5
1% boric acid	26.4	65.5	47.5	130.0	12.5	100.0	112.2	105.5
0.1% bromcresol purple	10.7	52.3	67.4	153.0	14.4	69.2	87.5	145.8
1% p-amino benzoic acid	13.0	29.5	24.6	102.5	11.0	64.0	51.0	242.0
1% butyl alcohol	36.8	64.5	119.0	92.5	7.0	55.0	49.0	98.0
1% caprylic alcohol	16.9	49.0	72.3	90.4	6.9	40.6	78.9	102.6
1% carbon tetrachloride	31.9	88.5	86.5	87.0	23.9	88.5	115.2	143.0
1% chlorobenzene	10.7	37.0	21.8	72.2	18.1	128.0	64.6	111.0
1% citric acid	1.6	8.7	59.0	79.4	1.1	7.7	58.8	110.0
0.1% cresol red	11.6	45.0	54.5	80.0	27.3	83.0	57.0	147.5
1% cyclohexanol	22.8	44.0	147.6	129.0	9.1	67.8	60.6	138.1
1% cymene	22.5	92.8	65.7	101.6	8.1	95.2	65.6	109.0
1% cysteine			141.4	69.0			38.6	97.8
1% diethylene glycol	30.0	94.0	73.1	169.0	8.7	41.7	68.4	140.0
1% 1–4 dioxane	16.1	71.5	80.0	122.5	11.0	87.4	82.0	90.6
0.3% formaldehyde	10.0	36.5	82.0	89.0	23.1	59.2	80.8	87.5
1% glycerol	16.8	73.4	38.0	77.3	16.0	84.1	124.0	87.9
1% hydrazine			88.6	57.8			76.5	78.6
1% hydroquinone	17.7	54.7	66.3	106.2	7.6	55.0	58.6	90.2
0.1% iodine	26.4	66.5	44.5	88.3	13.2	161.0	50.0	151.0
1% methanol	16.8	59.6	41.6	83.4	5.6	56.5	50.4	120.0
1% methyl iodide	28.4	112.5	141.8	135.8	7.5	36.6	53.2	77.2
1% methyl orange	16.8	86.2	33.4	76.5	7.2	25.2	56.2	103.9
1% magnesium nitrate	18.3	111.4	35.0	132.0	7.2	38.6	72.6	90.9
1% monopotassium phosphate	32.3	99.4	113.6	114.5	11.8	163.0	133.0	94.5
1% α-naphthol	18.2	71.0	56.2	98.0	8.3	37.8	75.8	145.8
1% ß-naphthol amine	25.6	70.8	39.8	46.7	16.4	94.1	86.6	99.0
1% para-formaldehyde	35.1	162.5	114.6	112.2	14.4	50.4	44.2	90.0
1% phenol	9.5	160.8	35.0	70.5	24.6	23.4	77.7	118.0
1% phthalic acid	14.1	86.5	87.0	138.8	1.9	13.5	96.6	130.4
1% potassium ferrocyanide	14.1	31.8	39.0	85.3	5.6	26.0	95.4	96.5
1% potassium thiocyanate	22.8	110.5	100.8	109.0	8.6	86.0	110.0	170.0
1% pyrogallic acid	13.4	26.8	30.1	109.5	4.0	57.0	62.8	99.3
1% resorcinol	9.6	44.5	45.0	97.8	17.1	82.2	109.2	119.5
1% rosaniline HCl	10.0	21.9	90.2	77.5	5.3	64.6	50.6	91.6
1% sodium benzoate	3.4	21.4	54.9	127.2	11.5	21.7	64.0	80.5
1% sodium hydrosulphite	23.8	105.1	94.3	116.5	9.5	100.0	61.2	124.8
1% sodium nitrite	15.7	85.5	67.0	71.6	9.4	83.1	42.0	77.5
1% sodium sulphite	32.9	105.0	35.5	93.0	39.0	83.0	32.4	41.4
1% toluidine	24.6	93.0	88.0	134.9	14.8	90.8	85.0	162.8
1% urea	12.8	48.5	114.0	111.8	7.0	64.8	82.5	106.8

[a] Numbers represent the average of the actual number of lesions per half-leaf obtained on 5 or more half-leaves of *Nicotiana glutinosa* or 12 or more half-leaves of *Phaseolus vulgaris* on inoculation with a purified virus preparation containing the designated chemical.

[b] Numbers represent the quotient of the average number of lesions per half-leaf obtained on 5 or more half leaves of *N. glutinosa* or 12 or more half-leaves of *P. vulgaris* on inoculation with the chemical-containing preparation, divided by the average number of lesions obtained on the other halves of the same leaves on inoculation with the control preparation.

TABLE 2.—*Infectivity tests on purified virus preparations immedately after the addition of various chemicals and after standing for 3 days at 5° C. Reagents in this group were found to cause an increase in the infectivity of the virus*

Chemicals tested	Tested immediately after mixing				Tested after 3 days at 5° C.			
	Phaseolus vulgaris		Nicotiana glutinosa		Phaseolus vulgaris		Nicotiana glutinosa	
	Chemical	Chemical ÷ control	Chemical	Chemical ÷ control	Chemical	Chemical ÷ control	Chemical	Chemical ÷ control
1% acetal	21.6[a]	67.1[b]	133.4[a]	153.0[b]	14.3	126.7	66.0	114.1
1% ammonium sulphate	26.0	155.5	55.5	80.0	20.3	157.0	94.5	116.0
1% benzidine	19.6	68.9	112.4	113.1	14.1	133.0	74.0	140.6
1% calcium chloride	16.1	47.5	143.0	138.8	10.9	39.6	100.2	148.0
1% carbon disulphide	41.6	94.5	110.0	111.0	9.2	124.0	91.5	118.0
0.1% cresolphthalein	40.2	146.2	60.1	119.8	27.2	115.1	32.8	139.0
1% furfural	48.0	123.5	121.8	120.5	15.7	159.0	60.2	292.0
1% guaiacol	4.9	48.0	44.0	146.5	11.3	45.1	70.8	127.2
1% l-menthol	38.4	88.0	110.6	106.0	20.4	115.8	133.6	210.0
1% phenolphthalein	12.7	270.0	75.0	113.5	44.4	139.5	127.0	197.2
1% phthalimide	31.9	198.1	22.5	100.0	21.8	122.4	73.2	145.6
1% potassium chlorate	43.6	203.0	99.4	133.1	28.9	161.5	59.8	238.3
1% dipotassium phosphate	102.4	317.0	120.2	104.8	34.2	713.0	87.8	130.9
1% pyridine	15.3	106.0	92.5	144.0	38.9	112.0	80.5	119.5
1% sodium nitrate	24.8	89.0	93.0	141.0	8.2	118.7	92.0	120.2
1% sodium thiosulphate	30.9	148.5	96.6	144.0	27.4	274.0	117.6	205.0
1% sulphur	17.5	156.1	147.2	111.0	46.2	218.0	44.7	137.0

[a] Numbers represent the average of the actual number of lesions per half-leaf obtained on 5 or more half-leaves of *Nicotiana glutinosa* or 12 or more half-leaves of *Phaseolus vulgaris* on inoculation with a purified virus preparation containing the designated chemical.

[b] Numbers represent the quotient of the average number of lesions per half-leaf obtained on 5 or more half-leaves of *N. glutinosa* or 12 or more half-leaves of *P. vulgaris* on inoculation with the chemical-containing preparation, divided by the average number of lesions obtained on the other halves of the same leaves on inoculation with the control preparation.

permanganate, potassium dichromate, iodine, and bromine, and the protein precipitants, such as mercuric acetate and chloride, silver nitrate, lead acetate, and safranine, were found to have a marked effect in reducing the number of lesions.

It seemed desirable to determine whether the reduction in the number of lesions was caused by action of the oxidizing agents and protein precipitants on the virus or on the plant used to test infectivity, or both. Table 4

TABLE 3.—*Infectivity tests on purified virus preparations immediately after the addition of various chemicals and after standing for 3 days at 5° C. Reagents in this group were found to cause a marked decrease in the infectivity of the virus*

Chemicals tested	pH	Tested immediately after mixing				Tested after 3 days at 5° C.			
		Phaseolus vulgaris		Nicotiana glutinosa		Phaseolus vulgaris		Nicotiana glutinosa	
		Chemical	Chemical ÷ control	Chemical	Chemical ÷ control	Chemical	Chemical ÷ control	Chemical	Chemical ÷ control
1% aluminum chloride	3.5[a]	0.9[b]	4.2[c]	15.6[b]	23.3[c]	0.6	2.3	14.6	14.8
1% aluminum sulphate	3.5	0.2	1.1	33.0	36.4	0.6	5.1	14.8	21.0
10% ammonium "		12.1	46.1	11.2	16.0	6.1	32.5	30.0	33.7
50% " "		2.5	10.8	3.6	3.7	1.1	5.7	8.4	11.4
1% " sulphide	9.0	45.6	226.0	68.2	87.5	0.6	7.7	18.8	55.5
1% barbital sodium	9.0	47.8	468.0	125.2	122.0	0.4	4.2	34.2	49.8
1% benzaldehyde	3.4	9.1	26.4	87.5	116.0	0.8	4.2	74.4	70.2
Saturated bromine	2.4	0.0	0.0	1.4	1.6	0.1	0.6	4.8	4.2
0.1% bromphenol blue	4.5	31.7	87.0	69.5	95.8	7.6	33.4	25.0	33.2
1% brucine	7.6	16.4	113.0	57.5	114.0	0.0	0.0	18.5	26.4
1% chloramine-T	6.4	0.0	0.0	2.7	13.7	0.0	0.0	0.0	0.0
1% cobalt acetate	6.6	19.2	52.7	94.4	69.2	21.2	58.2	67.2	77.1
1% " sulphate				15.6	28.4				
1% copper sulphate	3.7	0.5	3.1	0.6	0.9	0.5	4.0	15.7	14.4
1% cysteine HCl	1.8	0.2	0.5	24.5	64.8	0.0	0.0	25.9	28.2
1% diisoamyl amine	10.2	18.3	79.0	15.1	31.8	0.2	1.3	5.4	11.2
1% ferric chloride	2.2	1.9	73.0	28.8	55.5	0.0	0.0	0.0	0.0
1% ferrous sulphate	4.4	2.5	12.5	74.4	95.0	1.6	10.4	39.0	55.5
1% formic acid	3.1	1.6	5.0	46.5	35.8	1.3	11.3	20.8	36.6
1% hydrazine sulphate	1.5	3.4	7.0	20.0	36.7	0.0	0.0	6.4	5.6
3% hydrogen peroxide	6.2	1.1	3.4	8.2	7.2	4.4	12.9	19.2	14.2
1% hydroxyl amine HCl	3.8	1.5	8.0	37.3	91.0	0.8	5.1	19.6	29.6
1% iodine + 1% potassium iodide	6.2	0.0	0.0	2.0	2.4	0.4	3.2	1.4	1.4
1% lead acetate	5.8	6.6	19.6	83.8	84.6	1.6	21.9	63.0	68.4
1% " subacetate	7.1	3.4	23.1	65.5	87.0	0.7	4.9	4.6	10.6
1% mercuric acetate	4.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
1% " chloride	5.5	0.0	0.0	3.8	4.6	0.0	0.0	1.2	1.5
1% nickel sulphate				11.8	19.8				
1% oxalic acid	1.5	1.3	3.1	37.0	40.0	0.0	0.0	10.8	19.2
1% picric acid	1.7	5.7	14.1	36.8	48.0	0.0	0.0	0.4	0.7
1% platinic chloride	2.1	0.6	3.1	7.5	34.2	0.0	0.0	0.0	0.0
1% potassium cyanide	10.1	7.1	139.0	17.1	40.0	1.4	2.8	1.2	1.4
1% " dichromate		4.4	42.6	22.5	57.6	7.0	25.5	25.4	35.6
1% " ferricyanide	7.0	7.3	21.0	66.4	99.0	5.5	44.0	43.2	51.0
1% " permanganate	8.2	0.1	0.5	0.6	1.0	0.9	9.1	0.2	0.2
0.1% safranine	6.9	0.0	0.0	0.0	0.0	1.4	9.2	0.0	0.0
0.04% safranine	6.9	0.8	3.2	48.7	72.6	1.6	9.4	19.6	17.4
1% salicylic acid	3.1	0.3	0.6	6.2	26.2	0.1	0.4	30.4	46.9
1% silver nitrate	5.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
1% sodium sulphide	11.6	0.4	0.9	0.6	1.1	0.0	0.0	1.6	1.1
1% " tungstate	7.9	22.3	78.6	65.7	77.8	0.5	7.8	23.8	29.0
1% stannic chloride	1.1	0.4	0.9	0.8	1.2	0.0	0.0	0.3	0.4
1% stannous chloride	1.6	0.2	0.9	21.6	27.8	0.2	1.1	11.8	17.6
1% sulfanilic acid		1.1	26.2	0.1	0.6	0.0	0.0	26.2	44.1
1% tannic acid	4.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
1% tartaric acid	2.4	0.0	0.0	13.1	50.6	0.1	0.8	17.2	27.2
1% p-toluene sulfonic acid	1.6	3.3	16.3	15.6	21.4	0.1	0.7	1.6	3.1
1% zinc sulphate				4.6	9.2				

[a] Numbers represent the hydrogen-ion concentration of the chemical-containing virus preparations as determined using a glass electrode.

[b] Numbers represent the average of the actual number of lesions per half-leaf obtained on 5 or more half-leaves of *Nicotiana glutinosa* or 12 or more half-leaves of *Phaseolus vulgaris* on inoculation with a purified virus preparation containing the designated chemical.

[c] Numbers represent the quotient of the average number of lesions per half-leaf obtained on 5 or more half-leaves of *N. glutinosa* or 12 or more half-leaves of *P. vulgaris* on inoculation with the chemical-containing preparation, divided by the average number of lesions obtained on the other halves of the same leaves on inoculation with the control preparation.

records the results of an experiment in which chemicals were added to samples of a virus preparation and infectivity tests made after dialysis of the mixtures. A portion of each of the mixtures was dialyzed immediately after mixing and another portion dialyzed after standing for 3 days at 5° C. It may be seen that the reduction in number of lesions caused by oxidation is due to the action of the oxidizing agents on the virus, for after the removal of the chemical by dialysis the virus is inactive. Reversal of this inactivation by reducing agents has not been found possible, although inactivation by potassium permanganate may be interrupted by the addition of reducing agents such as cysteine. It appears that atomic oxygen is necessary in order to cause inactivation of the virus, for in an experiment in which oxygen gas was bubbled through a virus preparation by means of a sintered glass membrane, there was but a slight reduction in the infectivity of the virus after 8 days' treatment. Similar treatment with hydrogen gas for 8 days also caused but a slight reduction in the infectivity of the virus. Zinc dust at pH 3 was without effect, although sodium nitrite and sodium bisulphite at pH 3 caused a marked reduction in lesions. The two latter reagents had but slight effect at low hydrogen-ion concentrations, where the amounts of free nitrous and sulphurous acids were very small. It may also be seen from table 4 that, with the exception of mercuric acetate, the action of protein precipitants seems to be partly on the virus and partly on the plant used to measure infectivity. Cobalt, copper and zinc sulphates, potassium cyanide, and mercuric chloride appear to have but little direct effect on the virus, but a marked effect on the test plants. Silver nitrate required 3 days to cause a marked lowering of infectivity. The action of mercuric acetate, which was found to cause an immediate inactivation of the virus, was in marked contrast to that of mercuric chloride, which had but little effect even after 3 days. This may be due to the fact that mercuric acetate, which is more highly ionized than mercuric chloride, yields a greater number of reactive mercury ions. Because the fact that mercuric chloride is a powerful germicide, a detailed study of its action on virus was made.

In the first experiment, the effect of 0.5 per cent mercuric chloride on purified virus at hydrogen-ion concentrations from pH 3 to pH 8 was determined. To portions of purified virus at the various hydrogen-ion concentrations were added equal portions of 1 per cent mercuric chloride solutions at the same hydrogen-ion concentrations. Portions of each of the mixtures were immediately dialyzed against distilled water and then tested against a dialyzed control. Other portions were dialyzed at the end of various periods of time and similarly tested. The results of the infectivity tests are given in table 5. It may be seen that there is only a moderate inactivation of virus at pH 3, 4 and 5, even after long standing, but that at pH 6, 7, and 8 there is an immediate and practically complete inactivation

TABLE 4.—*Infectivity tests on purified virus preparations dialyzed after the addition of various chemicals. One portion of each of the preparations was dialyzed for 4 hours or more against distilled water immediately after the addition of the various chemicals and another portion was dialyzed after standing for 3 days at 5° C.*

Chemicals tested	pH	Dialyzed immediately after mixing		Dialyzed after standing for 3 days at 5° C.	
		Nicotiana glutinosa		Nicotiana glutinosa	
		Chemical	Chemical ÷ control	Chemical	Chemical ÷ control
1% aluminum chloride	3.5[a]	0.0[b]	0.0[c]	0.0	0.0
1% aluminum sulphate	3.5	0.0	0.0	0.0	0.0
1% chloramine-T	6.4	0.2	0.1	0.0	0.0
1% cobalt sulphate		40.2	73.3	51.4	125.5
1% copper sulphate	3.7	86.8	77.8	25.2	73.4
3% hydrogen peroxide	6.2	19.1	31.0		
1% iodine+1% potassium iodide	6.2	1.3	1.2		
1% mercuric acetate	4.1	0.0	0.0	0.0	0.0
1% mercuric chloride	5.5	94.0	66.7	34.3	70.6
1% nickel sulphate		48.9	82.3	15.0	29.3
1% potassium cyanide	10.1	132.6	91.2	117.9	90.5
1% potassium permanganate	8.2	0.0	0.0		
0.5% " "		0.0	0.0		
0.1% " "		17.2	45.8		
0.01% " "		38.9	65.0		
0.001% " "		45.0	83.1		
0.5% safranine	7.0	14.6	42.0		
1% salicylic acid	3.1			21.4	49.7
1% silver nitrate	5.1	29.9	78.0	14.6	22.4
1% sodium nitrite				116.7	74.5
1% sodium bisulphite				141.7	103.0
1% sulfanilic acid				26.2	76.1
1% tartaric acid	2.4			18.0	59.9
1% zinc sulphate				40.9	70.2
1% sodium nitrite[d]		20.1	16.1		
1% sodium bisulphite[d]		38.8	36.8		
1% zinc dust[d]		75.3	102.5		

[a] Numbers represent the hydrogen-ion concentration of the chemical-containing virus preparations before dialysis as determined by using a glass electrode.

[b] Numbers represent the average of the actual number of lesions per half-leaf obtained on 5 or more half-leaves of *Nicotiana glutinosa* or 12 or more half-leaves of *Phaseolus vulgaris* on inoculation with a purified virus preparation containing the designated chemical.

[c] Numbers represent the quotient of the average number of lesions per half-leaf obtained on 5 or more half-leaves of *N. glutinosa* or 12 or more half-leaves of *P. vulgaris* on inoculation with the chemical-containing preparation, divided by the average number of lesions obtained on the other halves of the leaves on inoculation with the control preparation.

[d] Preparations at pH 3 for 3 hours and then tested in the presence of the chemicals at pH 6.5.

TABLE 5.—*The effect of 0.5 per cent mercuric chloride on purified virus preparations at various hydrogen-ion concentrations over periods of time up to 4 months at 5° C.*

pH	Tested →	At once	After 1 week	After 3 weeks	After 4 months
3	Chemical	13.2[a]	8.4	21.8	0.3
	Chemical ÷ control	83.5[b]	29.2	76.0	4.9
4	Chemical	12.6	14.4	24.8	5.6
	Chemical ÷ control	45.0	34.4	86.0	61.5
5	Chemical	15.0	45.0	20.8	3.4
	Chemical ÷ control	78.9	64.6	72.9	40.0
6	Chemical	1.6	0.0	0.0	0.0
	Chemical ÷ control	7.3	0.0	0.0	0.0
7	Chemical	0.0	0.0	0.0	0.0
	Chemical ÷ control	0.0	0.0	0.0	0.0
8	Chemical	0.0	0.4	0.0	0.0
	Chemical ÷ control	0.0	1.4	0.0	0.0

[a] Numbers represent the average of the actual number of lesions per half-leaf obtained on 5 or more half-leaves of *Nicotiana glutinosa* or 12 or more half-leaves of *Phaseolus vulgaris* on inoculation with a purified virus preparation containing the designated chemical.

[b] Numbers represent the quotient of the average number of lesions per half-leaf obtained on 5 or more half-leaves of *N. glutinosa* or 12 or more half-leaves of *P. vulgaris* on inoculation with the chemical-containing preparation, divided by the average number of lesions obtained on the other halves of the same leaves on inoculation with the control preparation.

of the virus. The results of a similar experiment in which untreated juice from mosaic-diseased Turkish tobacco plants was used instead of purified virus are given in table 6. It may be seen that virus in untreated juice is only moderately inactivated and that the hydrogen-ion concentration appears to have but little effect on the amount of inactivation. The results of the two experiments suggest that, when virus is present with a large

excess of extraneous protein, as is the case with untreated juice, most of the mercuric chloride is used in combining with this extraneous protein, thus leaving but a small amount to act on the virus. Therefore, the virus is affected only moderately, regardless of the hydrogen-ion concentration. However, in the absence of a large amount of extraneous protein, as in the case of purified preparations, the virus is immediately and completely inac-

TABLE 6.—*The effect of 0.5 per cent mercuric chloride on virus in untreated juice at various hydrogen-ion concentrations over periods of time up to 3.5 months at 5° C.*

pH	Tested →	At once	After 3 days	After 1 month	After 3.5 months
3	Chemical	38.4[a]	60.6	107.5	13.8
	Chemical ÷ control	79.4[b]	71.4	76.4	33.8
4	Chemical	43.5	42.2	96.0	15.9
	Chemical ÷ control	70.4	72.3	67.2	59.6
5	Chemical	33.5	61.5	85.8	8.4
	Chemical ÷ control	66.5	68.6	50.0	33.6
6	Chemical	26.3	55.8	66.6	42.5
	Chemical ÷ control	68.2	95.0	45.5	64.1
7	Chemical	53.6	30.5	81.7	35.2
	Chemical ÷ control	87.8	66.5	56.2	68.0
8	Chemical	52.9	56.7	93.4	22.6
	Chemical ÷ control	54.3	75.0	50.9	67.0

[a] Numbers represent the average of the actual number of lesions per half-leaf obtained on 10 or more half-leaves of *Nicotiana glutinosa* or 12 or more half-leaves of *Phaseolus vulgaris* on inoculation with a purified virus preparation containing the designated chemical.

[b] Numbers represent the quotient of the average number of lesions per half-leaf obtained on 10 or more half-leaves of *N. glutinosa* or 12 or more half-leaves of *P. vulgaris* on inoculation with the chemical-containing preparation, divided by the average number of lesions obtained on the other halves of the same leaves on inoculation with the control preparation.

tivated at pH 6, 7, and 8. The protective action which is evidenced at pH 3, 4, and 5 must be due to the hydrogen-ion concentration. This suggests that the virus has an isoelectric point around pH 4.5, and that the protective action is due to the failure of the virus to combine with mercuric chloride at hydrogen-ion concentrations near the isoelectric point of the virus. This explanation appears all the more tenable in view of recent evidence that this virus is a protein (30).

It seemed of interest to determine the effect of large amounts of mercuric chloride on virus in purified preparations and also in untreated juice at pH 4.5. Table 7 gives the results of an experiment in which a 2 per cent solution and a 10 per cent suspension of mercuric chloride at pH 4.5 were added to equal volumes of untreated juice and a purified virus preparation at pH 4.5. Portions of each mixture were dialyzed at once and after standing for different periods of time at 5° C., and tested for infectivity. It may be seen that 1 per cent mercuric chloride had practically no effect on the infectivity of the virus in untreated juice, even after standing for 4 months, and 5 per cent mercuric chloride had only a moderate inactivating action. No bacterial growth was visible in the control preparation at the end of 1 month, although bacterial growth was visible at the end of 4 months. This probably accounts for the low infectivity of the control at the end of the 4-month period. All preparations containing mercuric chloride were without visible bacterial growth. It may also be seen from table 7 that 1 per cent mercuric chloride partially inactivates the virus in purified preparations, and that 5 per cent mercuric chloride almost completely inactivates the virus immediately after the addition of the reagent. It is believed that, although pH 4.5 is sufficiently close to the isoelectric point of the virus to render it inert toward small concentrations of mercuric chloride, it is not close enough to prevent interaction when large amounts of mercuric chloride are present. The action of tobacco-mosaic virus in the presence of mercuric chloride is consistent with the view that the virus is a protein.

Effect of Dipotassium Phosphate on Infectivity

The results of experiments on the effect of 0.1 M dipotassium phosphate on virus infectivity, which are recorded in table 2, indicate that this salt causes only a slight increase when the infectivity is measured on *Nicotiana glutinosa,* but that it causes a marked increase when the infectivity is measured on *Phaseolus vulgaris* variety Early Golden Cluster. Thornberry (32) found that dipotassium phosphate also caused a marked increase in the infectivity of tobacco-mosaic virus when tested on *P. vulgaris* variety Scotia. It seemed desirable to study the nature of the effect of phosphate on infectivity.

The results of an experiment designed to determine whether the effect

TABLE 7.—*The effect of 1 per cent and 5 per cent mercuric chloride on virus in untreated juice and in a purified preparation at pH 4.5 after standing for periods of time up to 4 months at 5° C.*

	Tested →		At once	After 1 week	After 1 month	After 4 months
Untreated juice	1% $HgCl_2$	Chemical	111.8[a]	77.2	73.7	25.2
		Chemical ÷ control	115.0[b]	86.5	98.5	157.0
	5% $HgCl_2$	Chemical	70.7	40.4	67.4	9.6
		Chemical ÷ control	88.4	45.3	37.7	71.0
Purified virus	1% $HgCl_2$	Chemical	57.2	43.0	75.4	13.3
		Chemical ÷ control	45.5	66.9	84.3	42.3
	5% $HgCl_2$	Chemical	2.7	0.0	0.0	0.0
		Chemical ÷ control	2.0	0.0	0.0	0.0

[a] Numbers represent the average of the actual number of lesions per half-leaf obtained on 10 or more half-leaves of *Nicotiana glutinosa* or 12 or more half-leaves of *Phaseolus vulgaris* on inoculation with a purified virus preparation containing the designated chemical.

[b] Numbers represent the quotient of the average number of lesions per half-leaf obtained on 10 or more half-leaves of *N. glutinosa* or 12 or more half-leaves of *P. vulgaris* on inoculation with the chemical-containing preparation, divided by the average number of lesions obtained on the other halves of the same leaves on inoculation with the control preparation.

of phosphate is due to the hydrogen-ion concentration changes that it causes or to some effect of the phosphate in itself are given in table 8. Portions of juice from diseased Turkish tobacco plants grown in a field were adjusted to different hydrogen-ion concentrations by the addition of equal portions of 0.1 M solutions of various buffers. In some cases it was necessary to add small amounts of 2 N HCl or NaOH in order to attain the desired hydrogen-ion concentrations. The solutions at the various hydrogen-ion concentrations were then tested for infectivity by the half-leaf method on plants of *Phaseolus vulgaris* and *Nicotiana glutinosa.* It may be seen from table 8 that, regardless of the buffer used, high hydrogen-ion concentrations cause a decrease in infectivity, which, as has already been pointed out, is considerable in the case of *P. vulgaris,* and low hydrogen-ion concentrations, within certain limits, tend to cause an increase in infectivity, which is also more marked in the case of *P. vulgaris.* At pH 5, 6, 7 and 8 the greatest increase in infectivity as measured on *P. vulgaris* occurred with

preparations containing phosphate. With one exception this was true when the infectivity was measured on *N. glutinosa,* although the increase was not nearly so marked as it was with *P. vulgaris.* There is some indication, therefore, that part of the increased infectivity in the presence of dipotassium phosphate is due to some action on the virus and part due to some action on the test plant.

TABLE 8.—*Effect of various buffers on the infectivity of tobacco-mosaic virus*

pH	Buffers	Phaseolus vulgaris	Nicotiana glutinosa
1	Phosphoric + hydrochloric acid	0.0[a]	0.0[a]
1	Hydrochloric acid	0.0	0.0
2	Hydrochloric acid	1.5	6.4
3	Citric acid	1.0	9.4
4	Sodium citrate + hydrochloric acid	4.4	7.2
4	Potassium phosphate + citric acid	3.8	8.2
4	Potassium acid phthalate	5.2	5.3
5	Sodium citrate + sodium hydroxide	11.6	10.2
5	Potassium phosphate + citric acid	19.7	12.8
5	Potassium acid phthalate + sodium hydroxide	12.5	14.7
5.5	Untreated diseased juice	35.9	10.4
6	Potassium acid phthalate + sodium hydroxide	20.5	10.3
6	Potassium phosphate + citric acid	73.5	26.1
6	Potassium phosphate	33.9	33.5
7	Potassium phosphate	50.1	31.0
7	Sodium veronal + hydrochloric acid	24.3	16.6
8	Sodium veronal + hydrochloric acid	59.3	28.5
8	Potassium phosphate	79.7	49.8
8	Sodium borate + sodium hydroxide	37.8	44.1
9	Sodium borate + sodium hydroxide	67.4	15.6
10	Sodium borate + sodium hydroxide	29.6	4.0

[a] Numbers represent the average of the actual number of lesions per half-leaf obtained on 20 or more half-leaves of *Phaseolus vulgaris* or 10 or more half-leaves of *Nicotiana glutinosa* on inoculation with the designated preparation.

An experiment was made for the purpose of determining the effect of different inoculation methods on the increase in infectivity caused by 0.1 M dipotassium phosphate and charcoal. Juice from diseased Turkish tobacco plants grown in a greenhouse was diluted with water and to portions of each dilution was added 0.1 gm. of charcoal (norite) per 10 cc. of the diluted juice. Another portion of juice was made up to 0.1 M phosphate by the addition of solid salt and then diluted with 0.1 M phosphate at pH 7. Plants of *Nicotiana glutinosa* were then inoculated with dilutions of the three preparations by rubbing and by needle puncture. It may be seen from table 9 that charcoal caused a marked increase in infectivity at all

TABLE 9.—*Effect of needle-puncture and rubbing methods of inoculation on the infectivity of tobacco-mosaic virus in the presence of dipotassium phosphate and charcoal*

Dilution	Preparation number	Rubbing inoculation			Needle-puncture inoculation		
		Untreated juice	Untreated juice +1% charcoal	Untreated juice +0.1M phosphate	Untreated juice	Untreated juice +1% charcoal	Untreated juice +0.1M phosphate
1:3	1	156.5_R[a]	203.5_L[a]		0.96_R[b]	2.32_L[b]	
		97.0_R		105.9_L[a]	0.91_L		0.51_R[b]
		119.6_L	211.9_R				
	2	101.5_L		99.5_R	1.52_R	1.7 L	
					1.56_L		1.12_R
	3	135.1_L	197.2_R		5.48_L		5.5 R
		157.5_L		190.0_R	4.5 R	6.2 L	
10^{-2}	1	63.4_R	167.9_L		0.48_R	0.96_L	
		63.4_R		53.8_L	0.6 L		0.48_R
		22.5_R		20.4_L			
		27.2_L	67.5_R				
	2	101.1_R		140.9_L	0.8 L	0.52_R	
					1.16_R		0.7 L
	3	99.0_L	230.9_R		1.8 R	2.1 L	
		121.5_L		155.2_R	2.1 R		2.4 L
10^{-3}	1	25.8_R	76.0_L				
		15.0_R		11.7_L			
		7.6_R		3.5_L			
		11.8_L	118.0_R				
	2	84.4_L		92.7_R			
	3	57.6_L	124.8_R				
		65.5_R		103.8_L			
10^{-4}	1	18.4_R	54.3_L				
	2	58.0_R		82.6_L			
	3	33.3_R	75.9_L				
		20.5_L		50.8_R			
10^{-5}	1	1.7_R	9.9_L				
	2	3.5_L		3.6_R			
	3	5.8_L	9.1_R				
		6.6_R		11.1_L			

[a] Numbers represent the average of the actual number of lesions per half-leaf obtained on 10 half-leaves of *Nicotiana glutinosa* on inoculation by rubbing of the designated preparation.

[b] Numbers represent the average of the actual number of lesions per half-leaf obtained on 50 leaves of *N. glutinosa* on inoculation of the designated preparation. Inoculation by needle puncture, 100 punctures per half-leaf.

R indicates inoculation was on right leaf-halves.

L indicates inoculation was on left leaf-halves.

dilutions with both methods of inoculation. Phosphate, however, caused either no change or a slight decrease in infectivity when the needle-puncture method of inoculation was used, and no appreciable change at low dilutions but an increase in infectivity at high dilutions when the rubbing method of inoculation was used. The experiment proves that phosphate does not cause an increase in infectivity under all conditions. Further demonstration of this fact was afforded in an experiment in which dilutions of untreated juice in 0.1 M phosphate at pH 7 in the presence and absence of charcoal were used to inoculate small Turkish tobacco plants. Dilutions of the juice with water were also tested in the presence and absence of charcoal. Each dilution of each preparation was inoculated to 50 small Turkish tobacco plants by rubbing the preparation once over one leaf of each plant. The results of the experiment, which are given in table 10, show that 0.1 M phosphate at pH 7 caused a decrease in infectivity at high dilutions, but that charcoal, even in the presence of phosphate, caused a marked increase in infectivity. Similar results were obtained in an experiment in which the inoculation was carried out by making 5 needle punctures on one leaf of each plant. As a whole, the results show that phosphate may have a variable effect on infectivity, depending on the dilution of the mosaic-diseased juice and on the plant used to measure infectivity. It may cause a decrease in infectivity in the case of Turkish tobacco plants, a decrease, no change, or an increase in the case of *N. glutinosa* plants, and a marked increase in infectivity in the case of *Phaseolus vulgaris* plants.

TABLE 10.—*Effect of phosphate and charcoal on the infectivity of tobacco-mosaic virus as measured on Turkish tobacco plants*

Dilution	Diseased juice diluted with water	Diseased juice diluted with water + 1% charcoal	Diseased juice diluted with 0.1 M phosphate at pH 7	Diseased juice diluted with 0.1 M phosphate at pH 7 + 1% charcoal
10^{-2}	48[a]	48	48	48
10^{-3}	48	48	45	48
10^{-4}	34	47	21	46
10^{-5}	7	32	1	34
10^{-6}	5	9	2	15

[a] Numbers represent the number of diseased plants out of 48 inoculated. Preparations rubbed once over one leaf of each plant.

Effect of Charcoal on Infectivity

Vinson and Petre (33) reported that charcoal caused an increase in the infectivity of tobacco-mosaic virus. Similar results were obtained in experiments in which virus was tested in the presence of phosphate and charcoal, the results of which are given in tables 9 and 10. Since little is known concerning the cause of this effect, it seemed desirable to study the action of charcoal in more detail. Table 11 records the results of an experiment in which a purified virus preparation was diluted with 0.1 M phosphate at pH 7 and various amounts of charcoal (Nuchar 00) were added to portions of each of the dilutions before inoculation to plants of *Phaseolus vulgaris*. It may be seen that from 0.01 per cent to 1 per cent charcoal by weight caused an increase in the number of lesions at all dilutions tested, with but 3 exceptions. However, 5 per cent charcoal still caused a marked increase in the number of lesions at a 1:10 dilution of virus, but at higher dilutions it caused a decrease in number of lesions, even preventing infection at the 1:100,000 dilution. Large amounts of charcoal caused a reduction in the number of lesions, and always prevented infection at high dilutions. Thus the effect of charcoal on infectivity may also be quite variable, causing an increase, no change, or a decrease in infectivity, depending on the relative proportions of virus and charcoal in the inoculum.

TABLE 11.—*Effect of different amounts of charcoal on the infectivity of a purified preparation of tobacco-mosaic virus*

Dilution of virus[a] →	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}
Virus	473[b]	234	81	11	2
Virus + 0.01% Nuchar 00	790	335	214	24	3
" + 0.1% " "	1052	381	296	33	1
" + 0.5% " "	1280	556	338	49	3
" + 1.0% " "	1457	735	215	6	2
" + 5.0% " "	1460	227	14	0.5	0
" + 10% " "	435	27	2	0	0
" + 20% " "	83	15	1	0	0

[a] Dilutions were made with 0.1 M phosphate at pH 7.

[b] Numbers represent the average of the actual number of lesions per leaf obtained on 14 leaves of *Phaseolus vulgaris* on inoculation with the designated preparation.

It appeared likely that a decrease in infectivity caused by large amounts of charcoal might be due to adsorption of virus on particles of charcoal. Several experiments concerned with the adsorption of virus on different

TABLE 12.—*Adsorption of tobacco-mosaic virus on charcoal*

	Phaseolus vulgaris				Nicotiana glutinosa			
Dilution[a] →	1	10	100	1000	1	10	100	1000
Virus (control) pH 6.7	157.5[c]	117.0	99.0	66.5	196.0[c]	173.1	104.3	31.0
Filtrate from (virus at pH 3 +10% Nuchar 00[b])	105.0	59.4	11.3	4.9	31.8	71.8	9.6	2.3
" " " " pH 5 " " " "	98.0	95.4	38.8	3.9	100.5	96.4	10.9	1.5
" " " " pH 6.7 " " " "	144.5	127.6	91.4	36.2	238.0	165.5	92.9	19.3
" " " " pH 8.1 " " " "	130.5	116.7	55.5	16.2	141.1	87.4	42.1	9.7
" " " " pH 9.2 " " " "	87.1	69.0	54.5	5.5	183.4	58.5	48.0	11.0
" " " +10% Nuchar XXX)	93.8	14.3	2.2	0.4	52.3	1.0	1.7	0.2
" " " " " " No. 2)	99.0	69.3	29.5	9.4	88.5	56.7	24.8	5.8
" " " " " " W)	127.1	97.0	57.2	9.3	113.2	121.8	19.0	14.4
" " " " " Norite)	159.9	146.0	100.9	17.5	161.2	130.0	49.0	17.5
" " " " " Nuchar 00)	144.5	127.6	91.4	36.4	238.0	165.5	92.9	19.3
" " (above " " "	3.0	0.7	0.4	0.2	3.0	0.3	0.3	0.0
" " " " " "	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Virus (control) pH 7.0	436.0[c]	394.0	205.0	71.5				
Filtrate from (virus + 10% Nuchar 00)	218.0	123.0	71.7	1.1				
" " (above " " "	1.8	0.0	0.0	0.0				
" " " " " "	1.1	0.0	0.0	0.0				
" " (virus + 5% " "	474.0	347.0	204.0	105.0				
" " (above " " "	72.3	27.2	3.9	0.8				
" " " " " "	1.4	0.2	0.0	0.0				
" " " " " "	0.0	0.0	0.0	0.0				

[a] Dilutions were made and then the charcoal was added.

[b] Preparations adjusted to pH 6.7 before inoculation to plants.

[c] Numbers represent the average of the actual number of lesions per leaf obtained on 12 or more leaves of *Phaseolus vulgaris* or 5 or more leaves of *Nicotiana glutinosa* on inoculation with the designated preparation and dilution.

kinds of charcoal under many different conditions were made. Purified and fairly concentrated virus preparations were used in most of these experiments. Unless otherwise specified, the dilutions were made with 0.1 M phosphate at pH 7 and the designated amount of charcoal was then added to each of the dilutions of the virus preparation. After stirring for 10 minutes, the mixtures were filtered through hardened filter paper. The amount of adsorption of virus on charcoal was judged by the infectivity of the filtrate. It may be seen from table 12 that adsorption of the virus on charcoal is at a maximum around pH 3 to 5, although some virus is adsorbed at all reactions tested. The results also indicate that more virus is adsorbed by the more finely divided charcoal preparations, such as Nuchar XXX, than by the coarser grades of charcoal. It may also be seen from table 12 that two treatments with 10 per cent by weight of charcoal, or 3 treatments with 5 per cent charcoal remove all of the virus from even fairly concentrated preparations. The results given in the first 4 rows of table 13 demonstrate that even 0.01 per cent to 0.5 per cent charcoal adsorbs a measurable amount of virus. The increase in the number of lesions at low dilutions of virus is probably due to the effect of small particles of charcoal that pass the filter paper. A striking demonstration of the effects that the same amount of charcoal may have on different amounts of virus is given in the 5th and 6th rows of table 13. When 1 per cent charcoal is added to a fairly concentrated virus preparation, the relative amount of virus adsorbed and removed on the charcoal on filtration is very small, so that the numbers of lesions produced before and after filtration are about the same. At a 1:10 dilution of virus, however, it may be seen that the filtrate from the 1 per cent charcoal gives only about one-half as many lesions as the preparation gave before filtration. At a 1:1000 dilution of the virus the adsorption and removal of the virus on the charcoal caused a reduction in lesions from 280 to 41, indicating that much of the virus had been removed on the charcoal. Thus, the net effect on the infectivity may vary widely, depending on the ratio between amount of virus and amount of charcoal. It may also be seen from table 13 that the first treatment with 10 per cent charcoal removed practically all of the virus, since, when this charcoal was resuspended and then diluted and tested, it gave many lesions even at a 1:10,000 dilution, whereas dilutions of the suspension of the second portion of charcoal gave only a few lesions. That virus in untreated mosaic-diseased juice may also be adsorbed and removed on charcoal is indicated by the results given in the last 3 rows of table 13.

DISCUSSION

Tests of the effect of a large number of chemicals on the infectivity of tobacco-mosaic virus have demonstrated that, with the exception of brucine, the only reagents that affect this virus directly are oxidizing agents, pro-

TABLE 13.—*Adsorption of tobacco-mosaic virus on various amounts of charcoal*

Dilution[a] →	1	10	100	1000	10,000	100,000
Virus (control) pH 7.0	889[e]	526	398	204	88.3	29.8
Filtrate from (virus + 0.01% Nuchar 00)		950	407	312	71.0	12.2
" " " + 0.1 % " "		991	466	129	14.2	3.4
" " " + 0.5 % " "		960	210	45	6.5	1.4
Virus + 1% Nuchar 00[b]	1448	1950	853	280	11.9	1.8
Filtrate from (virus + 1% Nuchar 00)	1367	1060	121	41	2.3	0.7
" " " + 5% " "		67	0.3	0.9	0.0	0.0
" " " + 10% " "	452	0.1	0.0	0.0	0.0	0.0
" " (above " " "	0.3	0.2	0.0	0.0		
Suspension of first 10% Nuchar 00 in 10 volumes 0.1M phosphate pH 7	2320	2310	673	373	76.4	
Suspension of second 10% Nuchar 00 in 10 volumes 0.1M phosphate pH 7	4.0	3.9	1.1	1.2	1.0	
Virus + 10% Nuchar 00[c]	602	374	167	24.9		
Untreated diseased juice[d]	63.4	26.2	2.4	0.4		
Filtrate from (above + 10% Nuchar 00[d])	2.0	0.2	0.0	0.0		
" " " " " "	0.0	0.0	0.0	0.0		

[a] Dilutions were made and then the charcoal was added.

[b] Charcoal not removed by filtration.

[c] Charcoal added and then dilutions were made. No additional charcoal used.

[d] Dilutions were made with water.

[e] Numbers represent the average of the actual number of lesions per leaf obtained on 12 or more leaves of *Phaseolus vulgaris* on inoculation with the designated preparation and dilution.

tein-precipitating agents, and materials causing a hydrogen-ion concentration known to inactivate virus. It has not been found possible to reverse the inactivation caused by oxidation by subsequent treatment with reducing agents, although inactivation caused by potassium permanganate may be interrupted by the addition of cysteine. However, the exact nature of the oxidation reaction has not been determined. The fact that this virus is precipitated by so many protein-precipitating agents is an indication that it is protein in nature. The action of salts of heavy metals, particularly mercuric chloride, is very significant. Proteins are relatively inert chemically at or near their isoelectric point, and are more reactive at hydrogen-ion concentrations removed from the isoelectric point. Mercuric chloride was found to affect virus to a markedly less extent at pH 3, 4, and 5 than at pH 6, 7, and 8. This suggests that this virus may be a protein having an isoelectric point near pH 4.5, thus rendering it inactive chemically toward mercuric chloride near pH 4.5, but allowing reaction with mercuric chloride at pH 6, 7, or 8. The fact that tobacco-mosaic virus was found to be unaffected over long periods of time by concentrations of mercuric chloride known (7, 23, 31) to be germicidal, indicates that it is not a bacterial organism. As a whole, the results are in harmony with the conception that this virus is a protein.

SUMMARY

The effect of 110 chemicals on the infectivity of purified preparations of tobacco-mosaic virus has been determined. Many chemicals were found that affected neither the virus nor the test plant. A few chemicals caused an increase in infectivity of virus. Many chemicals were found that caused a marked decrease in the infectivity of virus.

The necessity of differentiating between the action of chemical on virus and the action of chemical on the plant used to determine the infectivity of virus has been demonstrated.

In general, the chemicals that had a direct inactivating action on tobacco-mosaic virus may be classified as oxidizing agents, protein-precipitating agents, and agents causing a hydrogen-ion concentration known to inactivate the virus.

The fact that tobacco-mosaic virus was found to be unaffected over long periods of time by concentrations of mercuric chloride known to be germicidal is an indication that the virus is not a bacterial organism. Virus in purified preparations was affected by mercuric chloride at pH 6, 7, and 8 to a markedly greater extent than at pH 3, 4, and 5. These results are in accord with recent evidence that this virus is a protein.

Dipotassium phosphate or phosphate buffers near pH 7 cause a marked increase in the infectivity of tobacco-mosaic virus when tested on *Phaseolus*

vulgaris, but when tested on Turkish tobacco plants may cause a reduction in infectivity, and when tested on *Nicotiana glutinosa* may cause no change, an increase, or a decrease in infectivity.

Charcoal may cause no change, an increase, or a decrease in the infectivity of tobacco-mosaic virus, depending upon the ratio of amount of charcoal to amount of virus. Tobacco-mosaic virus may be completely removed from solution by adsorption on charcoal. Maximum adsorption occurs at pH 3 to 5 and with very finely divided charcoal.

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CHEMICAL STUDIES ON THE VIRUS OF TOBACCO MOSAIC

V. DETERMINATION OF OPTIMUM HYDROGEN-ION CONCENTRATIONS FOR PURIFICATION BY PRECIPITATION WITH LEAD ACETATE[1]

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Vinson and Petre (6), in 1929, found that the addition of small amounts of basic lead acetate to the juice of mosaic-diseased Turkish tobacco plants caused the precipitation of most of the pigment and protein without apparently removing or injuring the tobacco-mosaic virus. Two years later Vinson and Petre (7) outlined a procedure for obtaining virus from juice of diseased plants by precipitation with neutral lead acetate following a preliminary treatment with basic lead acetate to remove pigment. They reported that their preparations, which contained only about 1 per cent of the original solids content of the juice, had an infective power equal to that of the fresh juice, although they found that the discarded basic lead acetate precipitate contained as much as $\frac{1}{3}$ of the virus. Apparently they did not attempt to prevent the loss of virus during the basic-lead-acetate treatment. Because the colorless water-clear solutions which Vinson and Petre obtained with the lead-acetate method were indisputably very infectious and thus useful in many ways, and because of the possibility of improving the process in at least one of the steps, it was decided to make a detailed study of the purification of tobacco-mosaic virus with lead acetate. The present paper records the results of this study.

MATERIALS AND METHODS

The virus preparations used in the experiments to be reported consisted of the untreated juice from plants of mosaic-diseased *Nicotiana tabacum* L. variety Turkish grown in a field during the summer months. The plants were cut and kept in a freezing room for a few days and then allowed to thaw just before pressing. The juice was then filtered through 4 layers of bandage gauze.

Merck's reagent lead subacetate and neutral lead acetate were used for the preliminary precipitation of pigment and the precipitation of virus, respectively. Preparations containing 200 gm. of reagent per liter of solution were used. Determinations of hydrogen-ion concentrations were made by

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means of a MacInnes type glass electrode (2). Tests for virus infectivity were made on *Nicotiana glutinosa* L. and *Phaseolus vulgaris* L. variety Early Golden Cluster by the half-leaf method of inoculation previously described (1, 3, 4). It was found necessary, because of the variable effect of phosphate on virus infectivity (5), to dilute untreated diseased juice and the treated preparations with the same diluent whenever tests for infectivity were made. Unless otherwise specified, 0.1 M phosphate at pH 7 was used as a diluent, although in some instances, as a check, the phosphate was removed from the treated preparations by dialysis and then water was used as a diluent for these preparations and the untreated juice.

The 3 principal steps involved in the lead-acetate process consist of a preliminary precipitation with lead subacetate, the precipitation of virus with neutral lead acetate, and the elution of the virus from the latter precipitate. Preliminary experiments indicated that the amounts of lead subacetate and neutral lead acetate suggested by Vinson and Petre represented optimum quantities for average juice preparations. However, for preparations containing more than an average amount of protein, it was found that the relative amounts of these reagents should be increased, and for preparations containing less than an average amount of protein, it was found that the amounts should be decreased. The experiments in the present investigation were, therefore, confined to the determination of the optimum hydrogen-ion concentrations for carrying out each of the 3 principal steps in the process. This was done by 3 types of experiments. In the first the lead subacetate precipitation was carried out at various hydrogen-ion concentrations using separate portions of the same infectious juice preparation. The subsequent treatment of each of the portions was the same. In the second set of experiments the lead subacetate precipitation was carried out at the optimum hydrogen-ion concentration, as indicated by the first experiment. The preparation was then divided into several portions and the neutral lead acetate precipitation made at various hydrogen-ion concentrations. The subsequent treatment of each of the portions was identical. In the third experiment the two lead acetate precipitations were carried out at the optimum hydrogen-ion concentrations, as determined in the first two sets of experiments. A suspension of the neutral lead acetate precipitate was made and divided into several equal portions and these portions adjusted to various hydrogen-ion concentrations for elution of virus. The infectivities of the final preparations in each of the experiments were compared with each other.

EXPERIMENTAL

Four 200 cc. portions of untreated infectious juice were adjusted to pH 6, 7, 8, and 9, respectively, by the addition, with stirring, of from 2 drops to 2 cc. of 2 N NaOH. Then 6 cc. of a solution of lead subacetate containing

TABLE 1.—*Effect of various hydrogen-ion concentrations on the lead subacetate precipitation*

Dilution →		1		10		100		1000	
pH of precipitation		Phaseolus vulgaris	Nicotiana glutinosa	Phaseolus vulgaris	Nicotiana glutinosa	Phaseolus vulgaris	Nicotiana glutinosa	Phaseolus vulgaris	Nicotiana glutinosa
6	L[b]	10.8[c]	31.7[c]	12.4	43.4	2.0	12.0	1.2	6.0
7	R[b]	24.1	64.9	10.5	40.2	2.3	15.5	1.1	5.3
7	L	27.4	60.5	9.9	23.7	4.4	15.7	0.9	4.6
8	R	59.9	107.1	28.1	30.2	7.5	10.2	1.9	6.9
8	L	28.8	85.4	27.3	34.0	8.0	17.5	1.9	5.3
9	R	67.4	101.8	74.6	49.4	21.3	26.4	3.8	7.7
9	L	74.0	63.4	57.7	58.4	27.0	19.3	2.9	12.0
×[a]	R	65.3	60.6	48.7	47.6	24.1	17.4	6.6	18.5
×[a]	L	80.0	55.4	47.8	60.3	17.6	46.0	6.9	12.7
6	R	36.2	22.0	11.0	30.0	4.7	13.2	2.4	4.0

[a] The lead subacetate precipitation was omitted. Subsequent treatment same as other preparations.

[b] L and R indicate that inoculation was on left and right half-leaves, respectively.

[c] Numbers represent the average of the actual number of lesions on 10 or more half-leaves of *Phaseolus vulgaris* or 5 half-leaves of *Nicotiana glutinosa* on inoculation with the designated preparation and dilution.

200 gm. of reagent per 1 liter of solution was added, with stirring, to each preparation. The preparations were then centrifuged for 10 minutes and the supernatant liquids adjusted to pH 4.6 by the addition of from 1.7 to 3 cc. of 2 N HCl. A 5th 200 cc. portion of infectious juice was adjusted to pH 4.6 by the addition of 1.1 cc. of 2 N HCl and then 14 cc. portions of a preparation of 200 gm. of neutral lead acetate in 1 liter of solution were added to this, and to the other 4 preparations. After being stirred for 15 minutes, the 5 preparations were centrifuged for 10 minutes and the supernatant liquids from the cream-colored precipitates were discarded. Each precipitate was washed twice with 150 cc. of 0.3 M KH_2PO_4 and then suspended in 60 cc. of 0.1 M phosphate at pH 6.8 and stirred for 1 hour. The 5 mixtures were frozen overnight, then thawed, centrifuged and the supernatant liquids tested for virus activity. The results of the tests, which are given in table 1, show that much virus is lost when the lead subacetate precipitation is carried out at pH 6 or 7, and that but little virus is lost when the precipitation is carried out at pH 9. Precipitation at pH 9 is definitely superior to precipitation at pH 8. A comparison of the lesion counts for the preparations precipitated at pH 6 and 9 with those for the preparation in which the lead subacetate precipitation was omitted indicates that about 90 per cent of the virus is lost at pH 6, whereas the loss at pH 9 is less than 10 per cent. If, therefore, the procedure outlined by Vinson and Petre is modified to the extent that the lead subacetate precipitation is carried out at about pH 9 instead of at pH 6.5 as they recommend, a marked increase in the yield of virus may be obtained.

The effect of different hydrogen-ion concentrations on the neutral lead acetate precipitation was determined by adjusting 800 cc. of untreated infectious juice to pH 9.1 by adding 10 cc. of 2.3 N NaOH with stirring. Then 24 cc. of lead subacetate solution was added and, after stirring for 5 minutes, the preparation was centrifuged for 10 minutes and the supernatant liquid, now at pH 8.7, was divided into 4 equal portions. These were adjusted to pH 3, 4, 5, and 6 by the addition of 10, 5, 2.3, and 1.4 cc., respectively, of 2 N HCl, and then 14 cc. of neutral lead acetate solution was added to each, with stirring. After 15 minutes the 4 preparations were centrifuged and the supernatant liquids which were at pH 3.8, 4.6, 5.2, and 5.5, respectively, were discarded. The subsequent treatment of the 4 precipitates, involving washing with KH_2PO_4, freezing and elution with phosphate at pH 6.8, was exactly the same as that described in the first experiment. The 4 preparations were tested on leaf-halves against each other and also against a portion of the untreated infectious juice used as starting material. The results, which are given in table 2, indicate that the yield of virus is about the same whether the neutral lead acetate precipitation be carried out at pH 3, 4, 5, or 6. There is some slight indication that precipitation at pH 5 or 6 is pref-

TABLE 2.—*Effect of various hydrogen-ion concentrations on the neutral lead acetate precipitation of virus*

Dilution →		1		10		100		1000	
pH of precipitation		Phaseolus vulgaris	Nicotiana glutinosa	Phaseolus vulgaris	Nicotiana glutinosa	Phaseolus vulgaris	Nicotiana glutinosa	Phaseolus vulgaris	Nicotiana glutinosa
3	L[b]	81.5[c]	59.4[c]	30.7	59.2	13.2	27.2	2.4	13.4
4	R[b]	131.0	79.4	60.5	63.6	13.1	38.8	2.7	13.0
4	L	95.1	70.0	85.3	68.8	18.3	11.8	3.4	8.0
U.J.[a]	R	13.7	33.8	60.4	40.2	46.8	12.0	12.2	8.8
4	L	107.4	88.8	87.1	83.4	14.3	31.0	5.1	9.0
5	R	147.9	107.6	135.0	75.2	33.8	29.2	9.2	7.8
5	L	158.6	75.6	89.6	68.6	21.9	27.0	3.6	7.6
6	R	112.7	84.8	68.1	71.0	23.1	21.6	5.7	8.0
6	L	111.2	83.6	74.3	60.8	9.7	11.8	3.2	12.6
3	R	104.9	95.6	70.3	68.8	10.7	19.2	1.7	10.6

[a] Untreated infectious juice used for starting material. No chemical treatment.

[b] L and R indicate that inoculation was on left and right half-leaves, respectively.

[c] Numbers represent the average of the actual number of lesions obtained on 10 or more half-leaves of *Phaseolus vulgaris* or 5 half-leaves of *Nicotiana glutinosa* on inoculation with the designated preparation and dilution.

erable to precipitation at pH 3 or 4, so for routine preparations of virus the neutral lead acetate precipitation has been carried out at about pH 5.5.

The effect of different hydrogen-ion concentrations on the elution of virus from the neutral lead acetate precipitate was next studied. Twelve liters of untreated infectious juice was adjusted to pH 9.1 by the addition of 160 cc. of 2 N NaOH and then 360 cc. of lead subacetate solution was added slowly, with stirring. The mixture which was at pH 8.9 was centrifuged and the supernatant liquid adjusted to pH 5.5 by the addition of 120 cc. of 2 N HCl. Then 840 cc. of neutral lead acetate solution was added with stirring and, after standing for 30 minutes, the preparation was centrifuged and washed twice with 2 liters and once with 1 liter of 0.33 M KH_2PO_4. The precipitate was then evenly suspended in 1.2 liters of 0.1 M phosphate at pH 6.8 and divided into 6 portions of 200 cc. each. These portions were then adjusted to pH 4, 5, 6, 7, 8, and 9 by the addition of 6, 5.3, and 1.5 cc. of 2 N HCl and 1.5, 6.3, and 9 cc. of 2 N NaOH, respectively, and then frozen overnight. The next day the 6 preparations were thawed, centrifuged, the supernatant liquids removed, all adjusted to pH 7, and tested for infectivity. The results, which are presented in table 3, show that the optimum hydrogen-ion concentration for elution of virus from the neutral lead acetate precipitate is pH 7. A decreased yield of virus is obtained at pH 6, 8 and 9, and practically no virus is removed at pH 4 and 5. The optimum hydrogen-ion concentrations for carrying out the lead acetate process for purifying virus are, therefore, about pH 9 for the lead subacetate precipitation, about pH 5.5 for the neutral lead acetate precipitation, and about pH 7 for the elution of virus.

DISCUSSION

A greatly increased yield of virus may be obtained by modifying slightly the lead-acetate process for virus purification outlined by Vinson and Petre, by carrying out the lead subacetate precipitation at about pH 9 instead of at pH 6.5. Practically colorless, water-clear solutions of virus which are almost as infectious as the starting material may be obtained by this modified process. Virus preparations more infectious than the starting material may be obtained by reducing the volume of the phosphate eluent to less than that of the volume of the starting material. For example, preparations about 10 times as infectious as the starting material were obtained by reducing the volume of the eluent to 1/20 of that of the starting material. After such concentration, however, the preparations were always quite colored and also had a high solids content. Because of this, the modified lead-acetate process has not been found useful in preparing highly concentrated virus preparations. However, it has much to commend it as a method for preparing rap-

TABLE 3.—*Effect of various hydrogen-ion concentrations on elution of virus from the neutral lead acetate precipitate*

Dilution →		1		10		100		1000
pH of eluent		Phaseolus vulgaris	Nicotiana glutinosa	Phaseolus vulgaris	Nicotiana glutinosa	Phaseolus vulgaris	Nicotiana glutinosa	Phaseolus vulgaris
4	L[a]	1.3[b]	1.2[b]	0.4	0.0	0.0	0.0	0.0
5	R[a]	22.5	7.3	13.9	3.0	1.3	0.1	0.1
5	L	4.5	125.1	4.5	1.1	0.2	0.7	0.1
6	R	5.3	51.8	103.8	16.4	28.6	14.0	9.7
6	L	51.4	29.3	62.8	11.6	30.1	6.4	4.1
7	R	110.0	25.4	71.0	18.8	43.9	8.0	10.9
7	L	135.5	25.0	38.0	12.3	44.8	5.0	11.9
8	R	76.5	35.6	33.7	12.5	39.0	6.6	9.0
8	L	208.0	29.4	88.4	15.6	45.7	9.9	9.5
9	R	171.0	35.0	44.2	14.6	16.5	7.2	4.8
9	L	107.5	43.4	52.8	29.0	44.7	9.1	5.1
4	R	0.8	1.5	0.8	0.0	0.1	0.0	0.0

[a] L and R indicate that inoculation was on left and right half-leaves, respectively.

[b] Numbers represent the average of the actual number of lesions obtained on 16 or more half-leaves of *Phaseolus vulgaris* or 5 or more half-leaves of *Nicotiana glutinosa* on inoculation with the designated preparation and dilution.

idly small or even reasonably large amounts of fairly pure and moderately concentrated virus.

The modified process has been used routinely for the preparation of virus solutions used in the experiments reported in previous papers of this series. Infectivity tests on the partially purified preparations have demonstrated that a final yield of from 50 to 90 per cent of the virus in the starting material may be expected with the modified process. Experiments herein reported indicate that the original process outlined by Vinson and Petre results in a loss of about 90 per cent of the virus in the original juice. The greatest loss of virus occurs on the two lead acetate precipitates. Suspensions of the lead subacetate precipitates were always found to be infectious, although with the modified process the activity diminished rapidly on dilution, a 1:1000 dilution usually being noninfectious. The loss on the neutral lead acetate precipitate is due to incomplete elution and can be partially avoided by successive elutions of the precipitate. Many tests on the dialyzed supernatant from the neutral lead acetate precipitation demonstrated that it was, in most instances, practically, if not entirely, free of virus. Tests on the dialyzed KH_2PO_4 washes from the neutral lead acetate precipitate indicated that they contained either no virus or a very small amount of virus.

For routine use of the method, it is necessary that the starting material contain near a normal amount (2 to 4 per cent) of total solids. If a starting material is used that has been frozen, thawed and centrifuged several times and that has, as a result, a solids content of only about 1 per cent, then the lead subacetate precipitation will bring down most of the virus protein and the final preparation will contain practically no virus. If, on the other hand, the starting material contains 10 or 15 per cent total solids, the lead subacetate will fail to precipitate the pigment and the final preparation will contain a much greater percentage of inactive protein and will also be colored.

SUMMARY

The optimum hydrogen-ion concentrations for carrying out the 3 principal steps in the lead acetate process for the purification of tobacco-mosaic virus proposed by Vinson and Petre have been determined. The optimum hydrogen-ion concentration for the lead subacetate precipitation was found to be about pH 9, for the neutral lead acetate precipitation about pH 5.5, and for the elution of the virus from the neutral lead acetate precipitate about pH 7. A greatly increased yield of virus may be obtained by modifying their process slightly and carrying out the lead subacetate precipitation at about pH 9 instead of at pH 6.5. The modified process was found very useful for the rapid preparation of colorless partially purified solutions hav-

ing a virus concentration equal to or somewhat greater than that of the starting material.

FROM THE DEPARTMENT OF ANIMAL AND PLANT PATHOLOGY OF
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EFFECT OF TANNIC ACID ON THE INFECTIVITY OF TOBACCO-MOSAIC VIRUS

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(Accepted for publication May 4, 1935)[1]

Infectivity of tobacco-mosaic virus has been reported to be destroyed by certain chemicals and juices from certain plants. Whether destruction of infectivity resulted from inactivation of virus, from some effect on the host plant, or both, is not apparent. According to Stanley,[2] active trypsin prevents tobacco-mosaic virus infection by rendering the host plant non-susceptible. This protective action against infection occurred when trypsin was applied to the plants before or within 30 minutes after inoculation, but failed when the activity of the enzyme had been destroyed by the action of pepsin or by heat. Allard[3] reported that infectivity of tobacco-mosaic virus was destroyed in 5 days by tannic acid at a concentration of 1 part to 20 or 50 parts of juice and that it was almost destroyed in the same time by a concentration of 1 part of acid to 100 or 200 parts of juice. Unpublished data from previous experiments indicated that infectivity of virus at a 1×10^{-2} dilution, when measured by the local-lesion method on Scotia beans, was inhibited in 1 hour by a 1 per cent solution of tannic acid. Olitsky and Cox[4] reported that tannic acid prevented equine encephalomyelitis virus infection in mice when the acid was applied previous to intranasal inoculation, but had no effect on activity of the virus when applied 15 minutes after inoculation. The purpose of this paper is to report results obtained from experiments that were designed to show the effect of tannic acid on the infectivity of tobacco-mosaic virus.

EXPERIMENTATION

EFFECT OF TANNIC ACID ON BUFFERED VIRUS

Tobacco-mosaic virus at a 1×10^{-2} dilution in 0.1 molar phosphate buffers at reactions of pH 10.0, pH 8.5, pH 7.0, pH 4.5, and pH 3.0 was treated with tannic acid[5] at different concentrations. At the end of the desired exposure

[1] Published at the expense of The Rockefeller Institute for Medical Research out of the order determined by date of receipt of the manuscript.

[2] Stanley, W. M. Chemical studies on the virus of tobacco mosaic. I. Some effects of trypsin. Phytopath. 24: 1055–1085. 1934.

[3] Allard, H. A. Effects of various salts, acids, germicides, etc., upon the infectivity of the virus causing the mosaic disease of tobacco. Jour. Agr. Res. 13: 619–637. 1918.

[4] Olitsky, P. K., and H. R. Cox. Temporary prevention by chemical means of intranasal infection of mice with equine encephalomyelitis virus. Science n.s. 80: 566–567. 1934.

[5] Tannic acid used was Mallinckrodt's di-gallic acid c. p.

of virus to acid, infectivity was measured by rubbing the suspension on the primary leaves of 20 Scotia bean plants. Control plants were inoculated with the virus diluted to the same extent in the buffers. The number of local lesions produced from the various suspensions is given in table 1.

TABLE 1.—*Effect of tannic acid on the infectivity of buffered tobacco-mosaic virus at a dilution of* 1×10^{-2}

Concentration of tannic acid	Time virus exposed to acid	Virus diluted 1×10^{-2} in 0.1 M phosphate buffers				
		pH 10.0	pH 8.5	pH 7.0	pH 4.5	pH 3.0
Control		1617[a]	3468	3497	1605	1123
1.0 %	1 min.	475	512	245	28	30
"	5 "	395	172	97	9	8
"	10 "	134	204	48	3	4
"	15 "	121	196	39	0	0
"	30 "	135	93	31	0	0
"	60 "	0	0	0	0	0
0.5 %	" "	294	250	138	3	4
0.1 %	" "	2234	3217	1656	37	68
0.05%	" "	2587	3593	3420	551	553
0.01%	" "	3053	4467	4547	1891	1567
Check		0	0	0	0	0

[a] = Average number of lesions on 20 beans.

The data show that activity in an alkaline medium is inhibited by a 1 per cent solution of tannic acid in 1 hour and in an acid medium in 15 minutes. They also show that the amount of inhibition of infection depends upon the concentration of tannic acid and the time of action. Reduction of activity was greater in acid than in alkaline suspensions.

EFFECT OF TANNIC ACID ON NONBUFFERED VIRUS

Nonbuffered virus at various dilutions was treated with tannic acid at different concentrations and then inoculated to 20 Scotia bean plants without adjusting the reaction or the concentration of tannic acid. Control plants were inoculated with virus suspensions at comparable dilutions in distilled water.

The results (Table 2) indicates that activity, regardless of virus concentration, was inhibited in 15 minutes at room temperature by tannic acid in 1 per cent concentration, while virus treated for 1 to 10 minutes was slightly infectious. In solutions of less concentration of acid (0.5, 0.1, 0.05, and 0.01 per cent) virus infectivity, after an exposure of 1 hour, was inversely proportional to the concentration of acid. Virus exposed to a .5 per cent solution of the acid for 5 to 20 days was noninfectious. It is not

TABLE 2.—*Effect of tannic acid on the infectivity of nonbuffered tobacco-mosaic virus at various dilutions*

Virus dilution	Reaction of suspension	Concentration of acid	Time of action										
			1 min.	5 min.	10 min.	15 min.	30 min.	60 min.	6 hrs.	1 day	5 days	10 days	20 days
0.5×10^{-1}	pH 5.4	1.0%	18[a]	6	3	0	0	0					
"	"	Control (none)						12,390					
1.0×10^{-1}	pH 5.8	1.0%	12	9	2	0	0	0					
"	"	Control (none)						4,955					
1.0×10^{-2}	pH 6.2	1.0 %	106	15	11	0	0	0					
"	"	0.5 %	487	283	214	93	28	10	78	4	0	0	0
"	"	0.1 %	864	555	341	226	107	46	20	24	18	16	30
"	"	0.05%	1437	1269	1108	1235	1093	986	492	181	133	22	46
"	"	0.01%	2329	1896	1941	1637	1728	1894	748	855	453	780	384
"	"	Control (none)						1872	826	985	769	890	472
Check			0	0	0	0	0	0	0	0	0	0	0

[a] = Average number of lesions on 20 beans.

evident from the data whether virus was inactivated appreciably by tannic acid at concentrations of 0.1, 0.05, and 0.01 per cent, since, on standing, the infectivity of the control sample was reduced to about the same degree as the acid samples. The reactions of the suspensions were measured colorimetrically.

RESTORATION OF VIRUS ACTIVITY UPON REMOVAL OF TANNIC ACID

(a) *By Ultrafiltration.* Activity of tannic acid-virus suspensions was partially restored upon removing the acid by ultrafiltration. Ten cc. of a noninfectious tannic acid-virus mixture were passed through collodion membranes (28.96 mμ pore diameter) which retained the virus but not the acid. After passing 20 cc. of distilled water through the material on the membrane to remove the remaining tannic acid, the residues were taken up in 10 cc. of 0.1 molar phosphate buffers at a reaction of pH 8.5 and inoculated to beans.

TABLE 3.—*Infectivity of virus in the residues after removal of tannic acid from noninfectious virus-tannic acid suspensions by ultrafiltration*

Virus dilution	Reaction of suspension	Concentration of tannic acid	Activity of virus				
			Unfiltered suspension	Residue of virus treated with tannic acid			
				1 day	5 days	10 days	15 days
1×10^{-1}	pH 5.8	None (control)	36,500[a]	26,500	22,500	21,000	19,530
"	"	1. %	0	24,000	24,000	18,000	17,650
"	"	2. %	0	21,500	26,500	20,500	21,440
"	"	5. %	0	16,500	21,500	16,500	16,300
"	"	10. %	0	19,750	23,500	19,500	19,250
1×10^{-2}	pH 6.2	None (control)	3,386	2,500	.....	.....	.....
"	"	1. %	0	1,827	.....	.....	.....
"	pH 8.5	None (control)	3,542	2,115	.....	.....	.....
"	"	1. %	0	1,370	.....	.....	.....

[a] = Average number of lesions on 20 Scotia beans inoculated at pH 8.5.

Ninety, 81, 62, and 74 per cent, respectively, (Table 3) of the activity was restored upon removing the acid from virus diluted 1×10^{-1} after treatment with 1, 2, 5, and 10 per cent tannic acid for 1 day, and 73 and 64 per cent of the activity was restored from virus diluted 1×10^{-2} at reactions of pH 6.2 and pH 8.5 after standing for 1 day in a 1 per cent solution of tannic acid. The virus was not permanently inactivated after standing 15 days in 10 per cent tannic acid.

(b) *By Precipitation with Gelatin.* Activity of tannic acid-virus suspensions was partially restored upon removing the acid by precipitation with gelatin. Two cc. of a 5 per cent solution of gelatin was added to 10

cc. of nonbuffered virus diluted 1×10^{-2} in 1 per cent solution of tannic acid. The materials were thoroughly mixed and inoculated to beans after periods of from 1 to 60 minutes. Similarly, noninfectious virus was treated with gelatin in 0.5, 0.1, 0.05, and 0.01 per cent concentrations and allowed to stand 1 hour before inoculating to beans. Control plants were inoculated with virus diluted 1×10^{-2} in distilled water, in a 1 per cent solution of tannic acid, and in a 1 per cent solution of gelatin.

The number of lesions produced from the inoculations indicated that 15 per cent of the original virus activity was restored within 1 minute by gelatin at 1 per cent concentration. From 24 to 64 per cent of the activity was recovered in 5 to 60 minutes in 1 per cent gelatin, while 41, 5, 1, and 1 per cent, respectively, was regained in 1 hour by 0.5, 0.1, 0.05, and 0.01 per cent gelatin. Virus diluted 1×10^{-2} in 1 per cent solution of gelatin produced 1834 lesions and that in water 1995 lesions on 40 bean leaves. Virus in a 1 per cent solution of tannic acid was noninfectious. It is apparent that a noninfectious tannic acid-virus suspension is made active upon precipitation of the acid with gelatin, and that the recovered activity appears to be proportional to the concentration of gelatin.

Effect of Tannic Acid on Infection by Virus Applied Either Before or After the Application of Acid

Inhibiting effect of tannic acid on infection when virus and the acid are applied separately to plants was determined in the following manner. The upper surfaces of the primary leaves of 20 bean plants were sprayed with 10, 5, 2, 1, 0.5, 0.1, 0.05, and 0.01 per cent solutions of tannic acid previous to inoculation with virus diluted 1×10^{-1} and 1×10^{-2} in distilled water. Other plants were inoculated with comparable samples of virus and the inoculated surfaces sprayed within 1 minute with 10, 5, 2, 1, 0.5, 0.1, 0.05, and 0.01 per cent solutions of the acid. Control plants not treated with the acid were inoculated with comparable samples of virus.

The results (Table 4) show that tannic acid, when applied in 0.01 to 10 per cent concentrations to the host plant before inoculations, greatly reduced infection, but in no case completely inhibited it, and that the degree of inhibition was proportional to the concentration of the acid. When applied in the same concentrations to plants after inoculation, it inhibited infection to a less degree. Plants inoculated with virus diluted 1×10^{-1} developed about 20 to 50 per cent as many lesions as the controls. Plants inoculated with virus diluted 1×10^{-2} developed about the same number of lesions as the controls, except those treated with acid at 1 per cent or greater concentrations. Acid at these concentrations reduced the number of lesions about 25 to 50 per cent.

TABLE 4.—*Effect of tannic acid on infection by virus applied before or after the application of acid*

Dilution of virus	Concentration of acid	Plants sprayed with tannic acid		Check
		Before inoculation	After inoculation	
1×10^{-1}	No acid (control)	8100[a]	8100	0
	.01%	4450	4680	0
	.05%	865	3650	0
	0.1 %	76	4600	0
	0.5 %	23	4450	0
	1. %	15	3800	0
	2. %	11	2100	0
	5. %	4	1900	0
	10. %	3	1750	0
1×10^{-2}	No acid (control)	1639	1639	0
	.01%	1690	1735	0
	.05%	460	1920	0
	0.1 %	34	1286	0
	0.5 %	28	1320	0
	1. %	16	1185	0
	2. %	6	1092	0
	5. %	5	873	0
	10. %	6	941	0

[a] = Average number of lesions on 20 Scotia beans.

DISCUSSION

Inhibition of tobacco-mosaic virus infection by tannic acid appears to result, in part, from an interaction of virus and acid, since complete inhibition depends upon concentration of acid and time of action. However, a part of the effect may result from the action of tannic acid on the host plant. The mechanism of the action is not apparent, but some of the well-known properties of tannic acid (precipitation of soluble proteins, combination with proteins, and dehydration of emulsoid materials) suggest an explanation of the reaction. According to Kruyt,[6] dehydration of agar hydrosols by tannic acid results from the adsorption of hydrophobic tannic acid molecules on the surface of the hydrophilic agar particles and not from a chemical affinity of the acid for water. If the acid molecules were adsorbed on the surface of the infectious particles as on agar, the physical state of the virus might be altered sufficiently to prevent infection without any permanent change in the virus. Such a condition might be readily reversible upon removal of the acid. If the virus were protein in nature or its activity were associated with pro-

[6] Kruyt, H. R. Colloids. Trans. by H. S. van Klooster. Ed. 2. 286 pp. John Wiley and Sons, Inc., New York. 1930.

teins, combination of tannic acid and protein might be expected to change the infectious substances into a noninfectious one.

Since the measurement of virus depends upon infection, a phenomenon not clearly understood, it is difficult to conclude whether the inhibition of infection results from a change in the virus, in the protoplasm of the host plant, or both. The fact that infection occurs in the presence of 1 to 10 per cent tannic acid when inoculation is carried out within 10 minutes appears to be evidence that a part of the inhibition of infection results from a physical or chemical reaction between the virus and tannic acid. The reaction appears to be reversible, since the amount of regained virus activity after precipitation of the acid with gelatin depends upon the concentration of gelatin and time of action. Activity was also restored upon separating the acid from virus by ultrafiltration, but, since it required about 2 hours to complete this procedure, the rate of reactivation was not studied. Virus after standing for 15 days in 10 per cent tannic acid was capable of recovering its infectivity.

Inhibition of tobacco-mosaic virus infection by tannic acid differs from that by trypsin,[7] but is similar, in general, to the action of tannic acid on the virus of equine encephalomyelitis.[8]

SUMMARY

1. Inhibition of tobacco-mosaic virus infection by tannic acid depends upon the concentration of acid and time of action.

2. Activity is restored upon removal of tannic acid from the virus suspensions by precipitation with gelatin or by ultrafiltration. Restored activity was proportional to the amount of gelatin added and time of action. Virus, after standing for 15 days in 10 per cent tannic acid, was made active by filtering off the acid.

3. Inhibition of infection on plants treated with tannic acid before inoculation was proportional to the concentration of acid. Tannic acid at concentrations of 0.01 to 10 per cent had but slight effect upon infection when applied to the plants after inoculation.

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[7] See footnote 2.

[8] See footnote 4.

PARTICLE DIAMETER OF CERTAIN PLANT VIRUSES AND PHYTOMONAS PRUNI BACTERIOPHAGE[1]

H. H. THORNBERRY

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INTRODUCTION AND HISTORICAL REVIEW

Determination of particle diameters of viruses by ultrafiltration methods has been greatly aided the last few years by Elford's (6) type of collodion membranes and its modification by Bauer and Hughes (2). Following Elford's (6) method, MacClement and Smith (17) estimated the viruses of Johnson's tobacco mosaics 1 and 6, aucuba mosaic, and Hyoscyamus mosaic to have particle diameters of 15, 15, 40–50, and 150 mμ[2], respectively. Smith (19), using the same method, reported potato x and y viruses to have diameters 200 mμ and 250 mμ, respectively. He (19) also mentioned that tomato spotted wilt and tobacco ring spot viruses passed through membranes of 350 mμ pore diameter, but were retained by those of 194 and 220 mμ, respectively. Duggar and Karrer (5) by ultrafiltration methods with alcohol-ether collodion membranes reported tobacco-mosaic virus to have a diameter of 30 mμ. By ultracentrifugal analysis Bechhold and Schlesinger (3) found that tobacco-mosaic virus had a diameter of 50 mμ, and Waugh and Vinson (22), by coefficient-of-diffusion analysis, reported its diameter to be 10 mμ. The purpose of this paper is to report results obtained from experiments designed to determine particle diameters of certain plant viruses and of *Phytomonas pruni* (E. F. Smith) Bergy *et al.* bacteriophage.[3]

METHODS

Viruses from the following diseases were used:

Johnson's (14) tobacco mosaic 1 and 6.
Holmes' (11) masked tobacco mosaic.
Bewley's (4) aucuba mosaic of tomato.
Kunkel's (16) second attenuated strain of aucuba mosaic.
Jensen's (12) yellow tobacco mosaic 101, 102 and 7a.
Valleau's (21) yellow ring spot of tobacco.
" (21) green " " " "
Wingard's ring spot of tobacco described by Fromme, Wingard, and Priode (9).

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[2] mμ = millimicron, 1×10^{-9} meter.

[3] *P. pruni* bacteriophage was obtained from Dr. H. W. Anderson, University of Illinois, who kindly supplied unpublished data on the procedure for producing lysis of the bacteria.

Porter's (18) cucumber mosaic, the virus of which was designated as "cucumber virus 1."
Johnson's (13) ring spot of potato.
Stanley's[4] purified tobacco mosaic 2/19/35.

All the virus samples were obtained from plants of *Nicotiana tabacum* L. var. Turkish that had been infected 3 or 4 weeks with the given virus. Anderson's (1) *Phytomonas pruni* bacteriophage was obtained from broth cultures. Infected plants were macerated with a food chopper and the infectious juice frozen in test tubes until needed for the experiments. Because of differences in infectivity of the original juices the samples were diluted to different degrees in order to equalize, as far as possible, the infectivity of the suspensions used for ultrafiltration. A given amount of the infectious juice (number of ml. to give the desired dilution in 100 ml. of liquid), after being frozen and thawed on three occasions, was added to 20 ml. of nutrient broth. This mixture was diluted to 100 ml. and adjusted to 0.1 molar phosphate salt concentration and a reaction of pH 8.5. At this reaction a flocculent precipitate formed and settled upon standing. The supernatant liquid after complete sedimentation of the precipitate was carefully decanted and filtered through Seitz E. K. filter pads under 700 mm. of mercury-negative pressure. Ten ml. of the Seitz filtrate were then passed through the collodion membranes of various pore diameters under 1 atmosphere of positive pressure obtained by compressed nitrogen gas. Previous to ultrafiltration, 2 ml. of broth that had been filtered through a membrane of 20 mμ pore diameter were passed through each membrane to saturate the areas of high surface energy.

Virus activity of the various samples was determined by inoculation to appropriate test plants. That of concentrated samples was determined by inoculating aliquots diluted to the extent of giving less than 300 lesions per leaf and the actual number of lesions produced multiplied by the dilution value. The plants used for each virus are mentioned in a footnote to table 2. Before inoculating Turkish tobacco plants, the reaction of the samples was adjusted from pH 8.5 to 6.5. Other plants were inoculated at pH 8.5. A small amount of carborundum powder No. 320 was added to the ring-spot and cucumber-mosaic samples before inoculation to cowpeas. Turkish tobacco plants, inoculated with samples of Holmes' masked tobacco mosaic were macerated and the juice rubbed on Scotia bean leaves to determine the presence of virus in the symptomless plants. All the plants were washed with running tap water after inoculation.

The samples of bacteriophage used were obtained from 16-hour-old

[4] Most samples were taken directly from mosaic-diseased plants; but one sample, purified by the lead acetate procedure, was obtained from Dr. W. M. Stanley.

broth cultures of *Phytomonas pruni* that had been lysed by the bacteriophage for 18 hours at 35° C. and a reaction of pH 7.2–7.4. This suspension was adjusted to pH 8.5 in 0.1 molar phosphate buffer and passed through Seitz E. K. filter pads under 700 mm. of mercury-negative pressure. Ten ml. of the Seitz filtrate free of bacteria and active at a dilution of 1×10^{-5} were passed through a graded series of collodion membranes to determine the end point of ultrafiltration. Activity of the ultrafiltrates was determined by adding 5 ml. of each sample after being adjusted to pH 7.2–7.4 to 5 ml. of a 16-hour-old broth culture of the organism. Lysis, as evidenced by clearing of the suspension after 18 hours, indicated the presence of bacteriophage and was designated by ++ signs in table 2. Utrafiltrates that

TABLE 1.—*Membrane data for the calculations of pore radius*

Membrane number	Per cent acetic acid	Thickness of membrane cm.	Coefficient of viscosity of water at 22° C., C.G.S.	Volume of water passing, ml./sec.	Pressure of water during flow dynes/sq. cm.	Volume of pores, ml.	Radius of pores, mμ.
63	0.40	0.018	0.00961	0.03561	399,600	0.1029	46.44
61	0.80	0.018	"	0.019005	"	0.1015	34.16
87	1.00	0.022	"	0.006205	"	0.1141	22.50
85	1.10	0.020	"	0.006594	"	0.1337	19.48
73	1.15	0.018	"	0.004663	"	0.1015	16.92
81	1.20	0.017	"	0.003003	"	0.0931	13.39

failed to produce visible lysis, were passed through Seitz E. K. filter pads and added to 5 ml. of a fresh broth culture of the organism. Visible clearing of these cultures, designated by a + sign, indicated that the ultrafiltrates contained bacteriophage in too small a concentration for lysis. Ultrafiltrates that failed to produce lysis in the second culture were considered to be free of active bacteriophage particles and were designated by a 0 sign.

Preparation of Membranes

The membranes were prepared according to the formula given by Bauer and Hughes (2) since Mallinckrodt's parlodion was used as the source of collodion instead of "Nicol" used by Elford (6). Constituents of the stock solution were:

Parlodion	150 grams
Absolute ethyl alcohol	250 "
Anhydrous ethyl ether	750 "
Acetone	1150 "
Iso-amyl alcohol	575 ml.

TABLE 2.—*Ultrafiltration of a certain plant viruses and Phytomonas pruni through collodion membranes of graded pore diameters*

Infectious agents	Sample		Unfiltered suspension	Seitz filtrate	Ultrafiltrates through membranes, pore diameters in millimicrons						Check
	pH	Dilution			92.88	68.32	45.00	38.96	33.84	26.27	
Johnson's tobacco mosaic #1	8.5	$1. \times 10^{-1}$	101,200[a]	131,300	3,100	585	141	0	0	0	0
			5[b]	5		5	5	0	0	0	0
Johnson's tobacco mosaic #6	8.5	$1. \times 10^{-1}$	96,500[a]	143,000	1,310	425	85	0	0	0	0
			5[b]	5		5	4	0	0	0	0
Holmes masked tobacco mosaic	8.5	1.66×10^{-1}	85,400[a]	128,500	850	540	118	0	0	0	0
			5[c]	5		5	4	0	0	0	0
Smith's aucuba mosaic of tomato	8.5	1.25×10^{-1}	87,900[a]	126,000	970	320	119	0	0	0	0
			5[b]	5	5	5	5	0	0	0	0
			96,000[d]	124,000	1,500	235	71	0	0	0	0
Kunkel's aucuba mosaic	8.5	1.25×10^{-1}	93,300[a]	132,600	1,600	640	160	0	0	0	0
			5[b]	5	5	5	5	0	0	0	0
			102,500[d]	145,000	970	300	24	0	0	0	0
Jensen's yellow tobacco mosaic #101	8.5	2.5×10^{-1}	82,700[a]	125,000	240	123	12	0	0	0	0
			5[b]	5	5	5	4	0	0	0	0
Jensen's yellow tobacco mosaic #102	8.5	2.5×10^{-1}	79,800[a]	112,000	450	125	58	0	0	0	0
			5[b]	5	5	5	5	0	0	0	0
Jensen's yellow tobacco mosaic #7a	8.5	5.0×10^{-1}	9,700[a]	13,000	157	13	4	0	0	0	0
			5[b]	5	5	5	2	0	0	0	0
Valleau's yellow ring spot of tobacco	8.5	3.3×10^{-1}	5[b]	5	5	5	5	0	0	0	0
			24[e]	190	155	131	28	0	0	0	0

TABLE 2.—(*Continued*)

Infectious agents	Sample		Unfiltered suspension	Seitz filtrate	Ultrafiltrates through membranes, pore diameters in millimicrons						Check
	pH	Dilution			92.88	68.32	45.00	38.96	33.84	26.27	
Valleau's green ring spot of tobacco	8.5	3.3×10^{-1}	5[b]	5	5	5	5	0	0	0	0
Wingard's ring spot of tobacco	8.5	3.3×10^{-1}	1,206[e]	840	740	350	48	0	0	0	0
			5[b]	5	5	5	4	0	0	0	0
Porter's cucumber mosaic #1	8.5	3.3×10^{-1}	565[e]	870	190	122	35	0	0	0	0
			5[b]	5	5	5	5	0	0	0	0
Johnson's ring spot of potato	8.5	2.5×10^{-1}	12[e]	9	7	6	2	0	0	0	0
			5[b]	5	5	5	5	0	0	0	0
Stanley's purified tob. mos. virus 2/19/35	8.5	$5. \times 10^{-1}$	12,560,000[a]	11,845,000			853	459	28	0	0
Anderson's *P. pruni* bacteriophage	8.5	$5. \times 10^{-1}$	++[f]	++	++	++	+	+	+	0	0

[a] Average number of lesions on 40 Scotia bean leaves.
[b] Number of diseased plants from 5 Turkish tobacco plants inoculated.
[c] Number of plants containing virus from 5 Turkish tobacco plants inoculated.
[d] Average number of lesions on 15 *Nicotiana sylvestris* leaves.
[e] Average number of lesions on 40 Black Eye cowpea leaves.
[f] Lysis of both cultures of *Phytomonas pruni* by bacteriophage.

The stock solution of collodion, after the addition of an equal volume of a mixture of 1 part of alcohol and 9 parts of ether by weight, was divided into 200 ml. portions and the amount of glacial acetic acid, indicated in table 1, was added to regulate the pore size. After thoroughly mixing, each solution was poured into leveled (by spirit levels) glass cells, 40 cm. in diameter, similar to those used by Bauer and Hughes (2). After allowing the solution to evaporate 75 minutes at 22° C. in a room with still air containing 60 per cent relative humidity, the glass cells with partially solidified collodion were submerged in distilled water until the collodion loosened (about 15 minutes) from the glass. After washing the membranes for 14 days with 3 changes of distilled water per day, small discs 3.5 cm. in diameter were cut from the central portion of the collodion sheet, and stored in distilled water until needed for ultrafiltration.

EXPERIMENTATION

Calibration of Membrane Pore Diameter

Pore diameters of the various membranes prepared were calculated according to the formula given by Elford (6):

$$r - 2l\sqrt{\frac{2nq}{pv}}, \text{ where:}$$

r—radius of pores in cm.
l—length of capillary, thickness of membrane in cm.
n—coefficient of viscosity of water at temperature of measurement in C. G. S. units.
q—volume of water passing in ml./sec.
p—pressure producing flow in dynes/sq. cm.
v—total volume of pores (assumed equivalent to water content of membrane).

Data (Table 1) necessary for the calculation of pore radius by this formula are given for membranes near the end point of ultrafiltration. Pore diameter instead of radius is used hereafter in this paper.

End Point of Ultrafiltration and Estimation of Particle Diameter

The end point of ultrafiltration of each virus and the bacteriophage, the smallest pores that allow the infectious particles to pass, was determined by filtering the samples under controlled conditions through a graded series of membranes. Infectivity of the ultrafiltrates indicates the membranes that allowed the infectious particles to pass. Data for membranes near the end point of ultrafiltration are given in table 2.

The data show that all the viruses in these experiments passed membranes of 45 mμ pore diameter, but were retained by membranes of 38.96 mμ pore diameter when the samples were not purified. According to Elford (7) who mentioned that particles were not greater than 0.33 the size of the

pores through which they passed, each of the viruses is estimated to have a diameter of 15 mμ. Tobacco-mosaic virus in samples purified by the lead-acetate procedure and concentrated to the extent of being active at a dilution of 1×10^{-6} and *Phytomonas pruni* bacteriophage passed through membranes of 33.84 mμ pore diameter, but were retained by membranes of 26.27 mμ pore diameter. The diameters of these infectious agents under these conditions are estimated to be 11 mμ.

DISCUSSION

Ultrafiltration analysis according to these results affords no aid in the classification of plant viruses on the basis of particle size, since all the unpurified suspensions contained infectious particles of approximately the same diameters. Particle diameters of some animal and plant viruses as well as bacteriophages, however, have been reported by several investigators to differ in size when estimated by the ultrafiltration analysis. Whether the factors that affect the passage of virus through the membrane interstices were sufficiently controlled by other investigators to estimate the minimal diameter of each virus, or whether the factors were adequately controlled in these studies to show diameter differences is not apparent.

An attempt was made in these studies to keep the conditions during ultrafiltration and the procedure in preparing the samples uniform so as to minimize, as far as possible, differences that might affect the passage of virus through the membranes. Adsorption of virus to the negatively charged membranes was eliminated, or greatly reduced, by adjusting the suspension to a reaction of pH 8.5 at which the viruses probably are negatively charged. Streaming potential between the membrane and suspension during the flow of liquid was prevented by the presence of ionizable phosphate buffer salts. Areas of high surface energy in the membranes capable of holding virus were saturated with the surface-tension-reducing substances in broth previous to ultrafiltration and an attempt was made to counteract differences in capillary-active substance, if any, in the various virus juices by adding 20 per cent broth to the suspensions. Gelatin, sodium oleate, and saponin, substances that also possess surface-tension-reducing properties and facilitate ultrafiltration, were inferior to broth in aiding ultrafiltration.

Physical properties of the viruses prepared by different procedures might have influenced the ability of the particles to pass through membrane interstices of irregular shape and thus account for the discrepancy between the particle diameter values found by MacClement and Smith (17) and those obtained in these studies for aucuba mosaic. It is well known that differently prepared samples of proteins may possess distinct physical properties. Reaction of the medium according to Svedberg's (20) sedi-

mentation analysis of proteins affects the molecular weight or particle size of proteins that have a characteristic stable zone. When the acid border of the stability region is exceeded denaturation and aggregation take place, and when the alkaline border is exceeded disintegration occurs. Within the stable zone a change of reaction does not affect the particles, as is shown by sedimentation analysis. Results from previous filtration and infectivity studies on tobacco mosaic suggest that the virus in 0.1 molar phosphate buffer at a reaction of pH 8.5 is dispersed to a greater degree than in non-treated juice. Conditions that affect dispersion may influence the estimation of particle diameter by ultrafiltration methods. Whether the reaction and concentration of salt of the suspensions affect particle hydration and in this way influence the estimation of particle diameter is not apparent. Hydration influences the values of molecular diameter of substances measured by certain methods, according to Kunitz, Anson, and Northrop (15). Elasticity of oil-emulsion particles, according to Hatschek (10), determines their passage through membrane pores smaller than the particles when sufficient pressure is applied. Whether the virus particles possess sufficient elasticity to change in shape under pressures applied during ultrafiltration is not apparent. Concentration of virus affects the end point of ultrafiltration, according to Elford and Andrews (8), and may account for the discrepancy in results. Tobacco-mosaic virus purified by the lead-acetate procedure and concentrated to the extent of being active at a dilution of 1×10^{-6} passed membranes of 33.84 mμ pore diameters, while nonpurified virus active at a dilution of 1×10^{-4} was retained by these membranes.

SUMMARY

1. Thirteen plant viruses and virus strains in nonpurified samples were estimated by ultrafiltration analysis to have diameters of about 15 mμ. One of these, tobacco-mosaic virus, in purified preparations contained particles estimated to be 11 mμ in diameter.

2. Particles of *Phytomonas pruni* bacteriophage were estimated to be 11 mμ in diameter.

FROM THE DEPARTMENT OF ANIMAL AND PLANT PATHOLOGY OF
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CLASSIFICATION OF SOUTHERN CELERY-MOSAIC VIRUS[1]

W. C. PRICE

(Accepted for publication July 24, 1935)

A mosaic disease of celery was found in Florida in 1924 by Foster and Webber (3). It has since been studied extensively by Doolittle (1), by Doolittle and Wellman (2), and by Wellman (6, 7, 8), and is commonly designated as southern celery mosaic. In a recent paper Wellman (6) pointed out the similarities and differences between southern celery mosaic and certain other plant-virus diseases. His results led him to conclude that the virus of this disease is specifically different from any other heretofore described, and he classified it as celery virus 1. Although similar to the common cucumber-mosaic virus in many respects, it was considered to be distinct from this virus because of certain striking differences in symptomatology and host range, and because of its slightly higher thermal death point and greater ability to withstand aging *in vitro*.

Recently, the writer (5) has described a technique for the demonstration of a specific immune reaction to cucumber mosaic in *Zinnia elegans* Jacq. and has suggested the usefulness of the technique for plant-virus classification purposes. Because of the marked similarities between southern celery mosaic and cucumber mosaic, it seemed desirable to make a comparison of the two from an immunological standpoint. Experiments were undertaken for the purpose of determining whether or not zinnia plants infected with celery-mosaic virus would become specifically immune from infection with cucumber-mosaic virus. In addition, a comparison was made of symptoms produced by the two viruses in certain host plants.

IMMUNOLOGICAL RELATIONSHIPS

A yellow strain of cucumber-mosaic virus, referred to here and in previous publications (4, 5) as strain 6, produces such conspicuous necrotic primary lesions in *Zinnia elegans* var. Golden Gem Midget that inoculation with it affords a convenient test of immunity in this plant. It was recently shown (5) that plants mottled by any one of several different strains of cucumber-mosaic virus are immune from virus of strain 6, but that plants infected with unrelated viruses, such as tobacco mosaic, are not immune from cucumber-mosaic-strain-6 virus. It was likewise shown that zinnia plants infected with tobacco- or aucuba-mosaic viruses become immune from a necrotic-type tobacco-mosaic virus (strain 302A), but that similar plants infected with cucumber-mosaic or other viruses unrelated to that of tobacco mosaic are not immune from this necrotic-type virus.

[1] Published at the expense of The Rockefeller Institute for Medical Research out of the order determined by date of receipt of the manuscript.

The plan of the present experiments was to inoculate zinnia plants with virus of southern celery mosaic,[2] to allow them to become thoroughly mottled, and finally, after a period of 20 to 30 days, to inoculate them with virus of cucumber mosaic strain 6. A number of such experiments were conducted, all inoculations being made by means of the rubbing method. Table 1 presents the results of two experiments, which show that virus of southern celery mosaic completely protects zinnia plants against cucumber mosaic strain 6.

TABLE 1.—*Numbers of lesions produced in diseased and healthy plants of zinnia by inoculation with cucumber mosaic strain 6 and tobacco mosaic strain 302A*

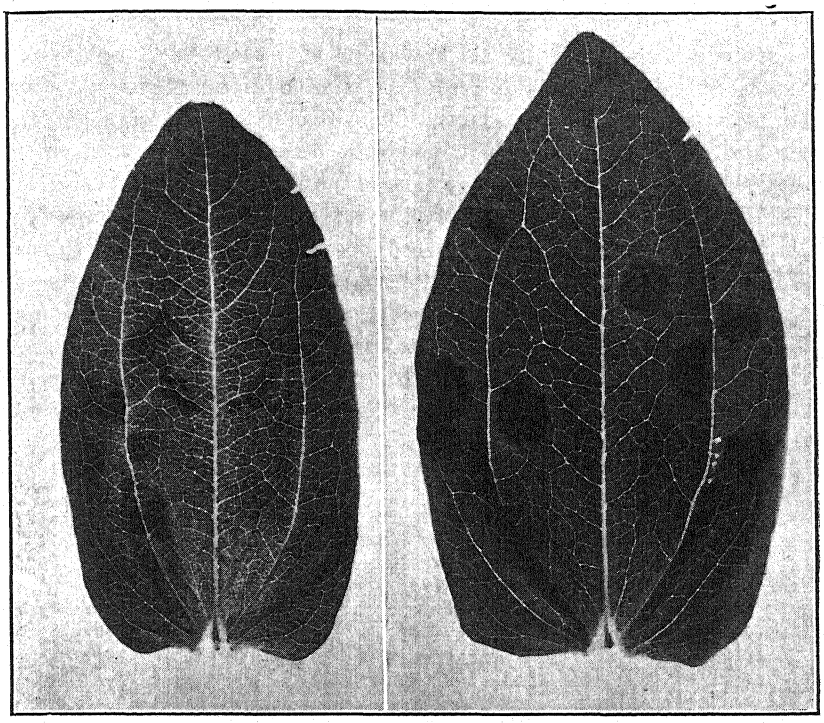
Plant No.	Lesions produced by inoculation with cucumber mosaic strain 6				Lesions produced by inoculation with tobacco mosaic strain 302A			
	Test 1		Test 2		Test 1		Test 2	
	Test plants	Controls	Test plants	Controls	Test plants	Controls	Test plants	Controls
1	0	86	0	5	7	39	23	46
2	0	51	0	1	0	51	38	42
3	0	26	0	2	0	16	14	63
4	0	50	0	5	7	16	42	30
5	0	46	0	3	4	20	61	27
6	0	25	0	2	7	36	45	57
7	0	23	0	2	11	10	12	36
8	0	36	0	3	3	5	23	56
9	0	65	0	2	4	57	5	63
10	0	51	0	14	29	17	17	28
Total	0	459	0	39	72	267	280	448

Figure 1 shows two zinnia leaves 7 days after they were inoculated with virus of cucumber mosaic strain 6. The leaf on the left was previously infected with celery virus 1, while that on the right was a healthy control. Necrotic lesions have developed only in the control leaf.

In another set of experiments, young zinnia plants were inoculated with celery-mosaic virus, allowed to become thoroughly mottled, and, finally, were inoculated with virus of tobacco mosaic strain 302A. The results of two experiments are given in table 1. They show that zinnia leaves mottled by celery-mosaic virus are not immue from infection with virus of tobacco mosaic strain 302A. It will, however, be seen from the table that fewer lesions appeared in the celery-mosaic-diseased plants than in the controls. This reduction in numbers of lesions is not specific but occurs when zinnia plants are infected with any one of a number of different viruses (5).

The results of the foregoing experiments show that zinnia plants infected with southern celery-mosaic virus become specifically immune from infec-

[2] A sample of southern celery-mosaic virus was kindly supplied by Dr. F. L. Wellman.



Photographed by J. A. Carlile.

FIG. 1. Leaves of *Zinnia elegans* Jacq. after inoculation with virus of cucumber mosaic strain 6. The leaf on the left was previously infected with southern celery-mosaic virus and shows the chlorois produced by this virus. It did not develop lesions when inoculated with virus of cucumber mosaic strain 6. The leaf on the right was a healthy control and has developed a number of necrotic lesions.

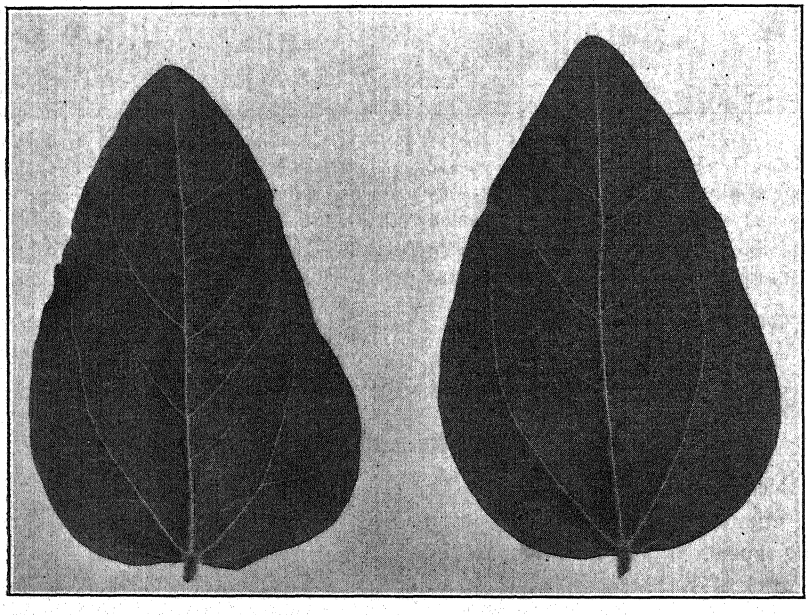
tion with virus of cucumber mosaic strain 6. It, therefore, is concluded that, from an immunological standpoint, celery-mosaic virus is closely related to cucumber-mosaic virus and that it should be classified as a strain of the latter.

HOST AND SYMPTOMATOLOGICAL RELATIONSHIPS

Because of the immunological relationship between celery-mosaic virus and cucumber-mosaic virus, it seemed of interest to examine more closely some aspects of the host relationships of these viruses. It is well known from the work of Wellman (6) that cucumber mosaic and celery mosaic have many characteristics in common. On the other hand, one of the criteria used by Wellman (6, 7, 8) for differentiating between celery mosaic and cucumber mosaic was the apparent difference in host range. While there has been no report of transmission of cucumber mosaic to hosts outside the Dicotyledoneae, it has been shown (8) that celery mosaic may infect at least 4 fam-

ilies of monocotyledonous plants. Moreover, certain of the dicotyledonous hosts of cucumber mosaic are not known to be susceptible to celery mosaic. It seems, however, not unlikely that this difference in host range may be due to the technique involved in determining susceptibility, or to some similar factor, and not primarily to a real difference between the two viruses. This has, indeed, proved to be the case in several instances.

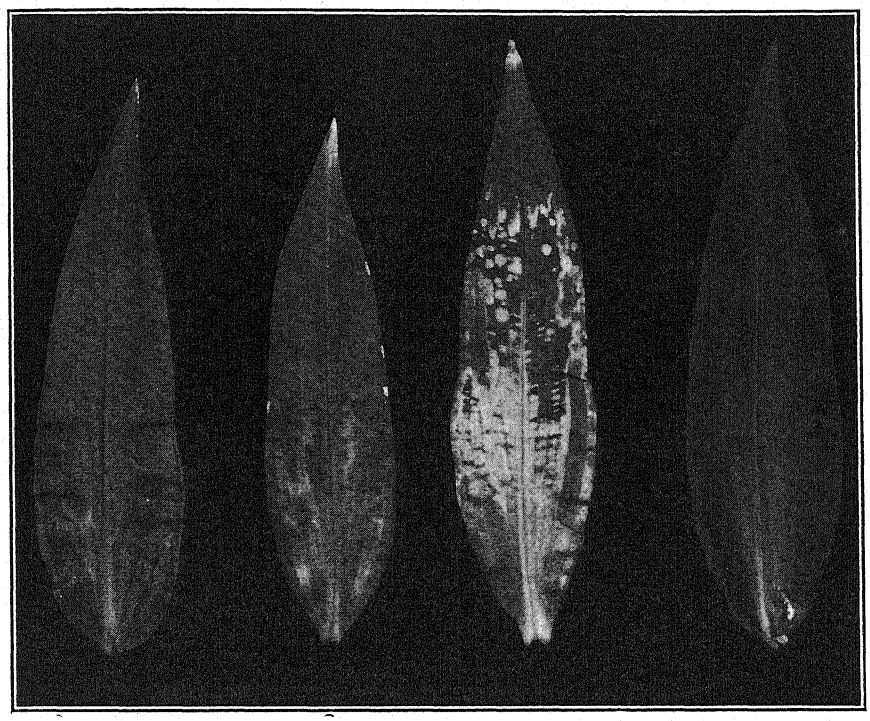
The symptoms of cucumber-mosaic virus in cowpea, *Vigna sinensis* (L.) Endl. var. Black, have been described previously (4). These consist of small, dark brown, necrotic, primary lesions in the inoculated leaves. The lesions appear within 2 days after inoculation and are not, as a rule, numerous. Although cowpea was listed by Wellman (8) as giving a negative response to inoculation with celery-mosaic virus, the writer has found the virus to be readily transmitted to the Black variety of cowpea by the rubbing method of inoculation. The symptoms produced consist of primary lesions that are slightly smaller than, but in other respects identical with, those caused by cucumber-mosaic virus. Cowpea leaves bearing lesions produced by cucumber- and celery-mosaic viruses are illustrated in figure 2.



Photographed by J. A. Carlile.

FIG. 2. Leaves of cowpea, *Vigna sinensis* (L.) Endl. var. Black, showing necrotic primary lesions produced by southern celery-mosaic virus (left) and cucumber-mosaic virus (right).

Wellman (8) reported transmission of celery mosaic to creeping day-flower, *Commelina communis* L., by means of *Aphis gossypii* Glover, but was unable to obtain transmission to this host by leaf-rubbing methods of inoculation. The writer, using the rubbing method of inoculation, has obtained infection of *C. communis* not only with celery-mosaic virus but also with ordinary cucumber-mosaic virus and with virus of cucumber mosaic strain 6. The symptoms produced are illustrated in figure 3. Porter's cucumber-mosaic virus causes a pale, greenish yellow, blotch-like mottling. The mottling induced by celery-mosaic virus is more prominent and of a more

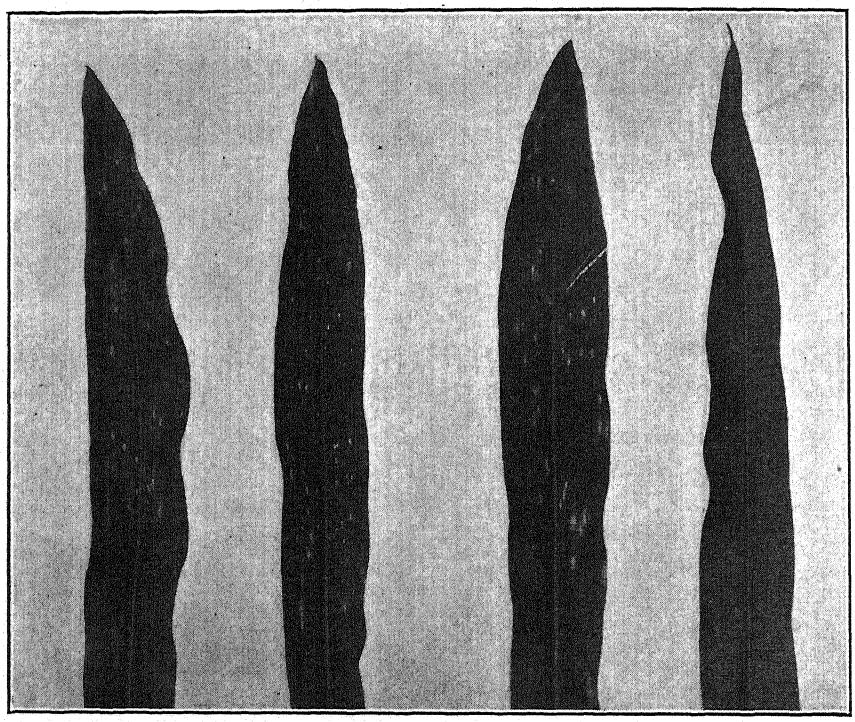


Photographed by J. A. Carlile.

FIG. 3. Leaves of *Commelina communis* L. (reading from left to right): from a plant infected with southern celery-mosaic virus, a plant infected with cucumber-mosaic virus, a plant infected with cucumber-mosaic-strain-6 virus, and from a healthy plant.

pronounced yellow. The mottling elicited by cucumber-mosaic virus strain 6 is still more brilliant and is associated with or preceded by the production of bright yellow rings. The symptoms produced by the 3 viruses differ principally in the intensity of the mottling pattern. Each of the viruses was recovered from systemically diseased leaves of *C. communis* by subinoculations to tobacco.

By means of the rubbing method of inoculation, using carborundum as an abrasive, cucumber-mosaic, cucumber-mosaic-strain-6, and celery-mosaic viruses were transmitted to corn,[3] *Zea mays* L. Elliptical, chlorotic lesions appeared in the inoculated leaves, soon became necrotic, and spread downward producing tan, necrotic streaks (Fig. 4). Systemic symptoms did not develop in any of the plants tested. There appears to be no consistent difference between the 3 strains of virus as regards the type of primary lesions



Photographed by J. A. Carlile.

FIG. 4. Leaves of field corn, *Zea mays* L., after inoculation from tobacco with (reading from left to right): Southern celery-mosaic virus, cucumber-mosaic virus, cucumber-mosaic-strain-6 virus, and with juice from a healthy tobacco plant. Similar necrotic primary lesions appear on leaves inoculated with each of the 3 viruses.

they produce in this strain of corn. Each of the 3 viruses was successfully reisolated by making subinoculations to tobacco from necrotic primary lesions 9 days after the original inoculation of corn.

The symptoms produced by cucumber- and celery-mosaic viruses in cow-

[3] An inbred strain developed by J. R. Holbert and designated as R4.

pea, *Commelina communis,* and corn suggest a close relationship between the two viruses.

DISCUSSION

The fact that there is a specific immune reaction between celery-mosaic virus and cucumber-mosaic virus shows that they are closely related and suggests that they should be classified as strains of the same virus. The virus of celery mosaic appears to be more infectious than that of ordinary cucumber mosaic. Its greater infectiousness might explain its apparently wider host range and its seemingly higher thermal death point and greater ability to withstand aging *in vitro.* The symptomatological differences between ordinary cucumber mosaic and celery mosaic are no greater than those characteristic of other well-recognized strains of cucumber mosaic. In the present paper it has been shown that *Commelina communis* and *Zea mays,* both considered to be immune from cucumber mosaic, are, in fact, susceptible to this disease. Furthermore, it has been shown that the cowpea, which was previously regarded as immune from celery mosaic, is, in fact, susceptible. These findings suggest that the host range of cucumber mosaic and celery mosaic may eventually be found to be identical. The evidence presented in this paper furnishes a striking example of the usefulness of immune reactions for the purpose of arriving at an understanding of plant-virus relationships.

SUMMARY

Infection of zinnia plants with southern-celery-mosaic virus induces in them a specific immunity from a yellow strain of cucumber-mosaic virus (strain 6). Celery-mosaic virus and cucumber-mosaic virus, therefore, are closely related immunologically and, it is believed, should be classified as strains of the same virus. Corroborative evidence is found in the fact that the symptoms produced by celery-mosaic virus in *Zea mays* L., *Commelina communis* L., and *Vigna sinensis* (L.) Endl. are similar to those produced by ordinary cucumber-mosaic virus in the same hosts.

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PHYTOPATHOLOGICAL NOTES

Peronospora in Storage Cabbage.—In 1926, Thung[1] reported on infection of full-grown cabbage heads by *Peronospora parasitica* (Pers.) de Bary in Holland and stated that this type of infection had not been mentioned in the literature. He also noted that the fungus was able to spread in the heads and sometimes affect the parenchyma of the stems in storage cabbage. Since that time Weber[2] has reported that all of the foliage leaves and the exposed head leaves of Florida cabbage in the field frequently are affected with downy mildew; but, so far as the writer has been able to determine, no mention has been made of the storage type of infection in this country. It is the purpose of this note to describe and illustrate a serious kind of peronospora infection of storage cabbage in Wisconsin.

On inspection of cabbage that had been in storage for about 4 months in a Milwaukee warehouse it was discovered that from 15 or 40 per cent of the stock in some bins showed badly discolored leaves and stems (Fig. 1, A, B, F). In some instances the grayish black discolorations suggested black rot, but on more careful examination it was apparent that this trouble was in the parenchyma tissues and not associated with the vascular elements, as in the case of black rot (Fig. 1, F, G). Furthermore, microscopic examination of free-hand sections of affected tissues revealed the presence of large haustoria within the cells. The relatively inconspicuous intercellular hyphae and the large haustoria were essentially identical with the description and illustrations given by Gardner[3] for *Peronospora parasitica* in storage turnips.

From the specimens studied it was apparent that the fungus had worked its way from affected lower leaves into the stem, followed the parenchyma tissues into the pith region, and from there spread upward into the head. In some heads the fungus reached the terminal bud and the smallest innermost leaves were diseased (Fig. 1, C–E). Elsewhere, along the length of the stem, the fungus occasionally spread out from the pith through the parenchyma into the midribs of the head leaves and caused serious grayish black discoloration of the midrib and large portions of the leaf blades (Fig. 1, A, B). The mycelium from such invaded leaves often came to the surface and, by contact, caused infection of the neighboring leaves. In the moist air spaces between the head leaves the fungus often produced a scanty mycelium and conidiophores bearing spores typical for this organism.

[1] Thung, T. H. *Peronospora parasitica* (Pers.) De By. attacking cabbage heads. Phytopath. 16: 365–366. 1926.

[2] Weber, G. F. Some diseases of cabbage and other crucifers in Florida. Fla. Agr. Expt. Sta. Bul. 256, p. 21. 1932.

[3] Gardner, M. W. Peronspora in turnip roots. Phytopath. 10: 321–322. 1920.

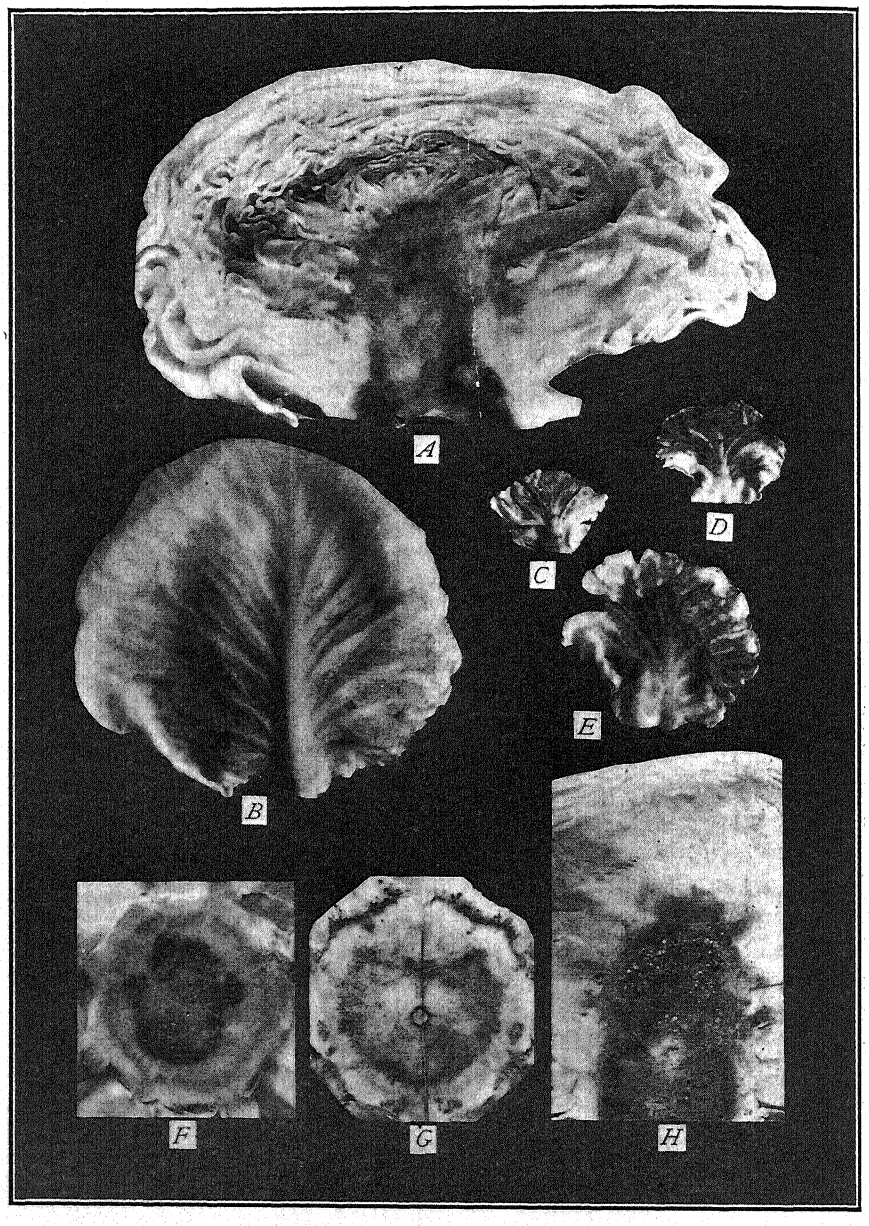


FIG. 1. A. Section through a cabbage head showing discolorations induced by *Peronspora parasitica*. B. Head leaf showing grayish black discoloration and breakdown of the blade tissues. C–E. Discolored innermost head leaves. F. Cross section of stem showing brown and black discoloration of the central cylinder. G. Cross section of stem showing discolored tissues outside the vascular ring which lead to the midribs of the outer head leaves. H. Longitudinal section of stem showing development of mycelium bearing conidiophores and conidia produced within 3 days in a humid atmosphere at 45°–50° F.

Small black spots, less than a millimeter in diameter, scattered over the surface of the leaves, suggested the possibility that they were the result of minor infections caused by germinating conidia. On the cut surfaces of affected heads placed in a refrigerator (45°–50° F.) typical conidiophores and conidia were produced within 3 days (Fig. 1, H). The conidia ranged from 22.5 to 30.0 μ in length and from 20.0 to 25.0 μ in width, the average size being 23.3×27.1 μ. No oospores were found in any of the affected heads of cabbage.

The seriousness of this disease as a storage and market trouble is readily apparent. In addition to the discoloration and decay of the tissues induced by Peronospora, the affected areas are very susceptible to secondary invasion by bacteria and such fungi as Alternaria and Rhizopus. In the storage lots under consideration great loss was occasioned by bacterial soft rot and alternaria rot following in the tissues affected by Peronospora. By excessive trimming some of the slightly affected heads were salvaged, but in many instances the salability of the stock was so affected that it was not worth the additional labor involved.

From the grower's standpoint it is significant that, in Holland, this fungus has been found to overwinter in old heads, leaves, and stumps of cabbage left in the field. Whether conditions in the cabbage-growing sections of Wisconsin permit the overwintering of the fungus in that manner is not known, but it is not unlikely that in some seasons it would be possible. The fact that this disease has not been reported in storage cabbage would indicate that either this type of infection is not common in northern-grown cabbage or it has been confused with other disease symptoms and not recognized as a separate disease. The potential seriousness of peronspora infection of full-grown cabbage warrants a thorough field and storage study of this fungus and the various types of disease symptoms induced.—G. B. RAMSEY, Bureau of Plant Industry, United States Department of Agriculture.

Toxicity of Low Concentrations of Ammonia to Mycelium and Sclerotia of Sclerotium rolfsii.—Since 1931 Southern sclerotium root rot caused by *Sclerotium rolfsii* Sacc. has been recognized as a serious disease of sugar beets in the Sacramento Valley of California. Measures for the prevention of distribution of the causal fungus and for the reduction of infestation by rotation are in operation, but satisfactory control of the disease is delayed by the absence of any effective method aimed directly at suppression of the fungus or prevention of infection.

Aqueous solutions of ammonia in concentrations as low as 50 ppm. of NH_3 for exposures of 24 hours have been found lethal to the mycelium of *S. rolfsii* in laboratory tests. Increasing the concentration to 150 ppm. of

NH_3 reduced the necessary period of exposure to 2 hours. The relation of concentration to the minimum lethal period is shown in accompanying figure 1. The minimum concentrations necessary to kill the more resistant

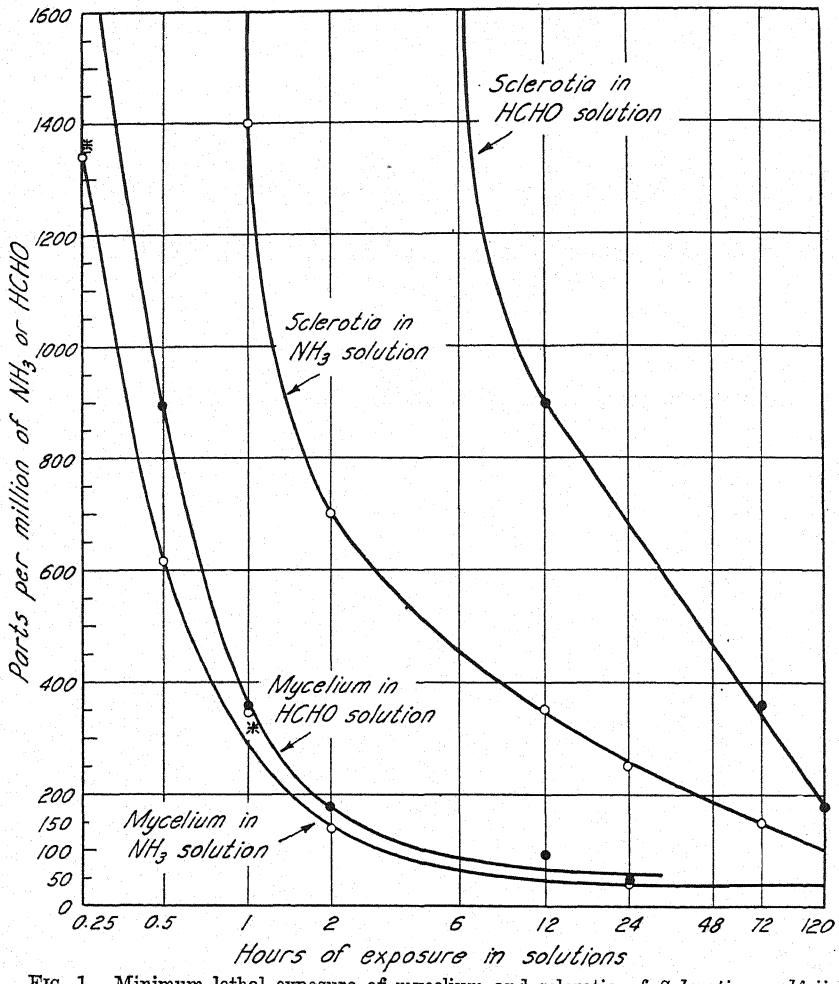


FIG. 1. Minimum lethal exposure of mycelium and sclerotia of *Sclerotium rolfsii* in solutions of ammonia or formaldehyde. Ninety-seven per cent of the mycelial masses were killed at the points indicated by the asterisks. All other exposures on which the curves were based were lethal to 100 per cent of the mycelial masses or sclerotia.

sclerotia within periods of 24 and 72 hours were, respectively, 250 and 150 ppm. of NH_3. In our tests formaldehyde required longer exposures than equal concentrations of ammonia to kill mycelium and sclerotia of *S. rolfsii*.

Previously, Neal[1,2] had found that mycelium of *Phymatotrichum omnivorum* was killed by 20 minutes' exposure in a solution containing 500 ppm. of NH_3. Sclerotia of the same fungus were killed by exposure for 20 minutes in 0.5 per cent of NH_4OH (approx. 2400 ppm. of NH_3).

The effectiveness of low concentrations of ammonia, as reported above, and the encouraging results of Streets[3] with heavy applications of ammonium sulphate and ammonium hydrate for the control of the cotton root-rot fungus on deciduous trees prompted applications of ammonium compounds on growing sugar beets in fields known to be heavily infested with *Sclerotium rolfsii*. Either anhydrous ammonia[4] or ammonium sulphate, dissolved in irrigation water in concentrations of approximately 300 ppm. of NH_3, significantly reduced the percentage of infection on sugar beets. The increase in yield of disease-free beets over nontreated plots was from 5 to 9 tons per acre and was more than sufficient to cover the cost of the treatments. Whether the beneficial results were due to the toxicity of low concentrations of ammonia in the soil or to the influence of the nitrogenous fertilizers upon the host plants has not been determined.—L. D. LEACH, AND A. E. DAVEY, Division of Plant Pathology, Branch of The College of Agriculture, Davis, California.

Blackberries: Possible Source of Streak Infection in Black Raspberries.—While making inspections during the summer of 1932 of bramble plantings entered for certification, 4 cases were found of a very severe streak infection on black raspberries when growing in close proximity to blackberries. In the literature on black-raspberry disease, nothing is found concerning the possibility of blackberries as a source of streak infection in black-raspberry plantings until Zundel[1] mentions it in 1934. Dodge and Wilcox,[2] in 1926, reported that streak may be found on blackberries, causing severe stunting. However, in neither case was any evidence presented to show the relationship between blackberries and black raspberries in the

[1] Neal, D. C., R. E. Wester and K. C. Gunn, Treatment of cotton root-rot with ammonia. Science n. s. 75: 139–140. 1932.

[2] Neal, David C., R. E. Wester, and Kenneth C. Gunn. Growth of the cotton root-rot fungus in synthetic media, and the toxic effect of ammonia on the fungus. Jour. Agr. Res. 47: 107–118. 1933.

[3] Streets, R. B. The treatment of deciduous fruit trees and nut trees infected by *Phymatotrichum omnivorum* with ammonium compounds. Science n s. 79: 417–418. 1934.

[4] Applied with apparatus furnished by and with the assistance of D. D. Waynick and F. H. Leavitt of the Association Laboratory, Anaheim, California.

[1] Zundel, George L., Raspberry diseases. Pennsylvania Agr. Exp. Sta. Ext. Circ. 133 (Revised), p. 18. 1934.

[2] Dodge, B. O., and R. B. Wilcox, Diseases of raspberries and blackberries. U. S. Dept. Agr. Farmers' Bul. 1488, p. 31. 1926.

spread of streak. Severe streak is characterized by a stunting of the growth, downward curling of the leaves, necrotic streaking of the new cane growth and production of a few seedy sour berries. On blackberries the symptoms become apparent during the latter part of May, often causing extreme stunting, but they usually do not appear on black raspberries until July.

Our records of Northwestern Pennsylvania show that in four cases in 1932, as well as one in 1933, blackberries were being grown adjacent to the black raspberries. Only mild infections of streak were found in any black raspberry plantings isolated 500 feet or more from blackberries.

One of the four cases mentioned as showing streaks is outlined in table 1. As will be noted, the first 2 rows are blackberries, while the balance of the planting is made up of black raspberries. In 1931 there was no streak found in either the blackberries or the black raspberries. In 1932, 1 per cent of streak was found in the blackberries and 30 to 60 per cent was found in the first two rows of black raspberries, 10 to 15 per cent in the next 2 rows, and 2 to 4 per cent in the last 2 rows. The plants for the Plum Farmer planting, set out in 1931, were taken from the planting set out in 1930. Plants from the 1930 Cumberland planting also were set out in 1931 on a neighboring farm, but were isolated from blackberries. This planting in 1932 showed one and one-half per cent of streak.

TABLE 1.—*Spread of streak disease in black raspberries beside blackberries on Young's Farm.*

	Variety	Per cent streak		
		1931	July 1 1932	August 1 1932
Rows 1 and 2, planted 1930	Eldorado blackberries	?	1	1
Rows 3 and 4, planted 1931	Plum Farmer black raspberries	0	30	60
Rows 5, 6, 7, planted 1930	Cumberland black raspberries	0	10	15
Rows 8, 9, 10, planted 1930	Plum Farmer black raspberries	0	2	4

Our records show that whenever a severe case of streak infection has been found in black raspberries, there always has been a planting of blackberries near-by. In one case we found the percentage of streak had increased from 6 per cent in 1931 to 30 per cent in 1932. In the adjacent blackberry planting there were a few black-raspberry mixtures that showed 100 per cent streak infection, while the blackberries themselves had approximately 1 per cent infection. On another farm, where black raspberries were being grown beside blackberries, the streak in 1931 was 5 per cent and in 1932 it had increased to approximately 75 per cent.

In another case there was a decided increase between the amount of

streak in black raspberries found in 1931 and that found in 1932. In this case no streak was found in the blackberries, and it could not be determined where the disease had its source. The percentage of streak found in black raspberries in all cases was always highest in the rows nearest the blackberries.

In 1933, a planting of black raspberries was found growing between two Eldorado blackberry plantings. All the black-raspberry plants were infected by streak but only one blackberry plant was suspected of being infected by streak.

Since no severe infection of streak was found in any black raspberry plantings isolated from blackberries and since the percentage of streak in black raspberries was always highest in rows nearest to blackberries, it is thought that, even in spite of the fact that blackberries often show little if any visible streak, there is a correlation involved that would bear deeper investigation.—J. K. THORNTON, State College, State College, Pennsylvania.

Susceptibility of Raspberry Species and Varieties to Leaf Spot (Mycosphaerella rubi) at Beltsville, Maryland.—The common leaf spot (*Mycosphaerella rubi* Roark) has been especially serious in a collection of raspberries at the U. S. Horticultural Field Station at Beltsville, Maryland, in 1934. Many varieties, including Viking, Lloyd George, and Newburgh, are not raised commercially so far south as Maryland because they are too susceptible to leaf spot to produce even a small crop there. The varieties represented in the collection at Beltsville are those that endure the conditions best and are actually somewhat resistant to leaf spot. However, such varieties as Latham and Chief were so badly affected that more than half their leaves were lost in 1934, and nearly all of the leaves of the variety June had dropped by the middle of September.

The relative resistance of the species and varieties is given in the following table. Some, listed in column 1 as more resistant than Latham and Chief, showed hardly a trace of leaf spot. In contrast, many varieties and a great many selections resulting from breeding work that are not listed in this table were more susceptible than Latham and Chief.

It should be noted that the 12 species listed are from eastern Asia and are highly resistant to leaf spot; in fact, no species from Asia so far tested has been found susceptible to leaf spot under conditions at Beltsville. It is of interest, also, that most Asiatic species tested are immune from or highly resistant to anthracnose and spur blight. There are no standard red or black raspberry varieties among those listed in column 1 as most resistant, but there are several little-known varieties of both in this list. It seems evident that resistance may be obtained both through breeding within the species, as indicated by breeding these resistant American varieties, as well as by hybridizing American with Asiatic species.

Hardiness of American varieties in Maryland is largely dependent upon resistance to leaf spot. When leaf spot is epidemic and the canes nearly defoliated as early as August or September, food reserves are not accumulated in sufficient quantity to make the plants hardy and the canes are more

Susceptibility of raspberries to leaf spot at Beltsville, Maryland, September 24, 1934

More resistant than Latham	As resistant as Latham and Chief	Highly susceptible, more than Latham
Rubus biflorus	Bristol-black	Black Beauty-black
" *incisus*	Dundee-black	Black Giant-black
" *innominatus*	Golden King-black	Cumberland-black
" *inopertus*	Naples-black	Variety from Kansas-black
" *Kuntzeanus*	Potomac-purple	Farmer-black
" *mesogaeus*	U.S.D.A. 5, 7-red	Honeysweet-black
" *parvifolius*	N.Y. 5371-red	June-red
" *niveus*	N.Y. 6523-red	Madison-black
" *phoenicolasius*	N.Y. 8126-red	New Logan-black
" *rosaefolius*	N.Y. 14443-red	Perfection-black
" *veitchii*		Quillen-black
" sp. F.P.I. 69115		Royal-purple
Van Fleet (*Kuntzeanus* × *Cuthbert*)		White Queen-red
Variety from Arkansas-red		
F.P.I. 75145-red		
U.S.D.A 0, 8, 9, 11-red[1]		
N.Y. 2609-black[2]		
N.Y. 5555-red		
N.Y. 5621-red		
N.Y. 5670-red		
N.Y. 10723-purple[3]		
N.Y 10724		
N.Y. 14619-red		

[1] Red = derived from *R. strigosus, R. idaeus,* or hybrids of these two species.

[2] Black = *R. occidentalis.*

[3] Purple = hybrids of red and black varieties.

readily injured by winter cold. Leaf spot was epidemic in 1933 as well as in 1934, and it is for this reason that in most sections east of the Allegheny Mountains Latham showed severe winter injury last spring from central New Jersey southward, wherever this disease was not controlled by spraying.—GEORGE M. DARROW, Bureau of Plant Industry, U. S. Dept. of Agr. Washington, D. C.

Sclerotinia Wilt of Canada Thistle.—During the summer of 1932 attention was called to the dying of Canada thistle (*Carduus arvensis* (L.) Robs.) near Craig, Colorado, and several large patches of the thistle were kept under observation.

A fungus was consistently isolated from the diseased plants and identified as *Sclerotinia sclerotiorum* (Lib.) Massee, after making host-range tests and comparing with a known culture of *S. sclerotiorum*. Both cultures reacted similarly on potato, tomato, bean, lettuce, celery, sunflower, and Canada thistle plants. Working with the same fungus, Young and Morris[1] observed that Canada thistles commonly were killed when growing close to wilting sunflowers.

Canada thistle plants completely wilted within 5 days after inoculation with the fungus. The cankers on the roots have a rather soft consistency, gray to dark brown, and of a water-soaked appearance. The parenchyma tissue in the diseased area is decomposed, leaving only the lignified tissues

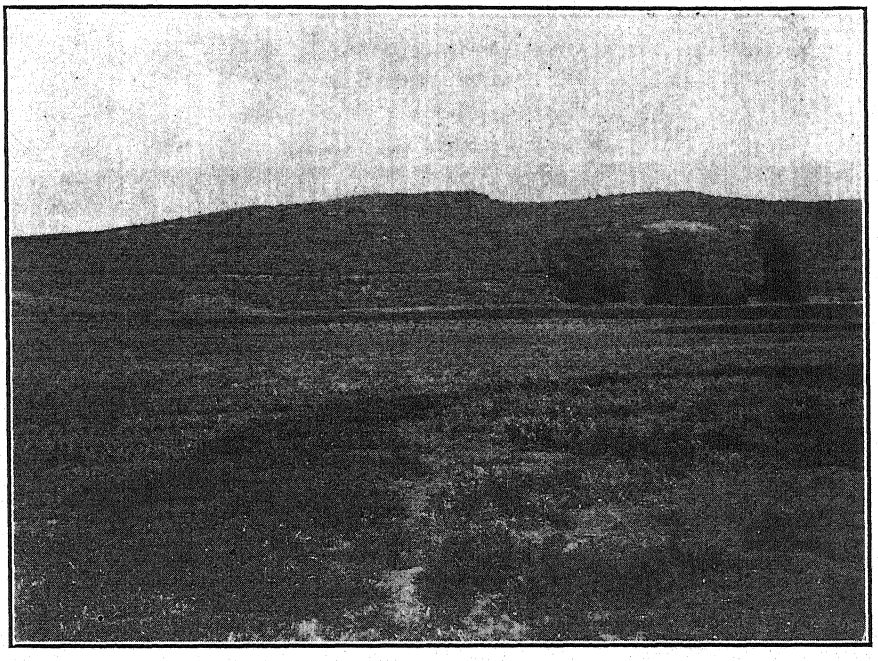


FIG. 1. Part of a large patch of Canada thistle growing near Craig, Colorado, affected with *Sclerotinia sclerotiorum*. The advancing outer edge of the patch is seen to the left and the dying central area to the right.

[1] Young, P. A., and H. E. Morris. Sclerotinia wilt of sunflowers. Montana Agr. Exp. Sta. Bul. 208. 1927.

and giving the root a shredded appearance. Irregular, hard, black sclerotia are produced both on the outside and in the cavities of the diseased stem.

Because of the rapidity and severity of attack, use of the fungus was suggested as a possible means of controlling the thistle. By the summer of 1934, however, it was found that the organism did not completely destroy the thistle. Though large patches were nearly destroyed, a vigorous fringe of the thistle kept ahead of the fungus and a "fairy ring" type of growth resulted (Fig. 1). As *Sclerotinia sclerotiorum* is not completely effective and because of its wide host range, its use as a biologic control agent is out of consideration.—E. W. BODINE, Colorado Agricultural Experiment Station, Fort Collins, Colorado.

REPORT OF THE 1935 ANNUAL MEETING OF THE SOUTHERN DIVISION OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

The annual meeting of the Southern Division of The American Phytopathological Society was held in the Henry Grady Hotel, Atlanta, Georgia, January 31–February 1, 1935, in conjunction with the Association of Southern Agricultural Workers.

The attendance was unusually large, there being from 30 to 35 present at each of the 3, half-day sessions, and among these were pathologists from 10 States.

At a business meeting the following officers were elected:

President—G. F. WEBER, University of Florida, Agricultural Experiment Station, Gainesville, Florida

Secretary-Treasurer—J. C. MCKEE, Mississippi State College, State College, Mississippi

Councillor—(reelected) V. H. YOUNG, University of Arkansas, Agricultural Experiment Station, Fayetteville, Arkansas

Thirty-five papers were presented. Twelve papers dealt with diseases of fruit and vegetable crops, 11 with cotton diseases, and 12 with diseases of miscellaneous crops. January 31 was devoted to discussions of cotton in its various aspects by the different Sections comprising the Association, and on that day the phytopathologists held a symposium on cotton-disease problems that occupied a morning session. Representatives were present from most of the important cotton-producing States and an interesting and mutually helpful discussion of the cotton-disease problems was given.

Other papers were those relating to diseases of strawberries, vegetable crops, and tobacco.

Dinners and entertainment, which were thoroughly enjoyed by all who attended, were given for the Association on January 30th by the Chilean Nitrate Educational Bureau, Inc., and by the Agricultural Development Bureau of the Barrett Company on January 31st.

EUGENE C. TIMS, *Secretary-Treasurer*

A Rhizoctonia Bud Rot of Strawberry.—A. N. BROOKS.

Rhizoctonia bud rot of strawberries in Florida occurs during the cool, moist weather of November to March. Although not widespread, and, therefore, of minor importance, it does cause severe damage in individual fields. The disease symptoms are dry rots of leaf- and flower-buds, and bases of petioles and stipules, the last causing the older leaves to lose their upright position and lie flat on the ground. Death of affected plants sometimes results. Recovery takes place through the development of adventitious buds. Under favorable conditions of low temperature and abundant moisture any aboveground plant part in contact with the soil may develop the rot. Fruit in

any stage of maturity is subject to this rot, which is probably the same fruit rot described by Dodge and Stevens. The roots and rhizomes are not affected. The causal organism belongs to the group represented by *Rhizoctonia solani*. Healthy plants in sterile soil readily become diseased when inoculated with sclerotia of this rhizoctonia either by placing the sclerotia in the buds or in the soil. The environmental conditions necessary for the rapid development of bud rot are temperatures below 75° F. and relatively high humidities, together with high organic soil content. Spraying with Bordeaux mixture is ineffective and likewise undesirable because of the fruit on the plants at the time the disease develops. The possible means of control seem to be (1) lowering of the organic content of the soil by reduction in summer cover crops; and (2) supplying adequate air drainage about the plants by greater spacing of the plants on the beds, or by later setting of plants so as to maintain a bush of smaller size.

Sclerotium rolfsii Sacc. on Strawberries and the Effect of Certain Chemicals on the Sclerotia.—R. E. NOLAN.

During the past 3 years *Sclerotium rolfsii* has occurred on strawberry plants in the Plant City area, especially during the warm months. All parts of the plant may be attacked, although often only one part, the bud, the fruit, or the roots, is found severely infected. The lower leaves of infected plants lie close to the ground. The lower part of the petiole and stipules generally shows the first signs of infection. The fungus later invades the bud, quickly killing it. Adventitious buds may or may not be formed later. Occasionally, during wet periods in the spring, newly set plants are attacked. The root systems of such plants are covered with a mass of white mycelium, and the roots are light brown. After death, sclerotia sometimes form. During the fruiting season an irregular, light brown soft spot may be found on the soil side of some of the small berries. As the berry grows the spot becomes larger, until ripening occurs. An abundant quantity of the white mycelium is then produced, completely covering the berry and causing a soft rot. When the berry is consumed the mycelium disappears, leaving a mass of sclerotia and strawberry seed on the surface of the soil.

Inoculation experiments show that a saturated atmosphere quickens the germination of the sclerotia and subsequent infection. In tests conducted to date to determine the effect, formaldehyde and carbon bisulphide killed all sclerotia, while benzol, paradichloralbenzene, chloroform, ether, and ammonia, as well as xylol, permitted varying amounts of germination.

A Bark Disease of Tahiti Lime Trees Caused by Phomopsis citri Fawcett and Diplodia natalensis Evans.—W. B. TISDALE.

Tahiti lime trees planted in Florida often die during the first 4 years after they are set in groves. Some trees die back quickly from the pruning cuts; others make vigorous growth for 2 or 3 years before they show signs of disease. The decline is often chronic. Infection of larger trees is manifested by a yellowing of the foliage and dead areas of bark on the trunk or large branches. The wood under the dead bark also is invaded and is usually darker than normal. Gum frequently exudes from the invaded areas. Salmon pink spore masses of a Fusarium and pycnidia of a Diplodia are frequently observed on dead areas on the trunks of large trees.

Several fungi have been isolated from the invaded parts of trees of different ages, but *Phomopsis citri* and *Diplodia natalensis* have been obtained singly or in combination in a majority of cases. Phomopsis occurs more frequently in diseased young trees and small branches, while Diplodia is associated more frequently with affected

trunks and large branches of older trees. Both organisms have been highly pathogenic in inoculation tests. Only a few of the other organisms isolated have shown any signs of parasitism under similar conditions.

Anthracnose Disease of Eggplants.—T. D. PERSONS.

On October 25, 1934, specimens of eggplant fruits showing a fruit rot were received from Sanatorium, Mississippi. One fruit showed 2 different types of lesions and examination with the microscope revealed that 2 different fungi were responsible. The fungus in one of the spots rather closely resembled the description given of *Gleosporium melongenea*. The fungus found in the other was entirely different as to spore size and shape. Cultures of this fungus were made and it was found to resemble closely a culture of a Collectotrichum isolated from Lima beans some time ago. The Lima bean fungus recently has been referred to *Colletotrichum truncatum* (Schw.), n. comb., by C. F. Andrus of the U. S. Department of Agriculture. A review of the literature on eggplant diseases has failed to disclose a species of Colletotrichum of this description on eggplant. Accordingly, preliminary inoculation experiments were begun in order to determine whether the eggplant fungus was the same as the bean fungus. Readings were taken about 10 days later and it was found that the lesions produced on the young bean stems by the eggplant fungus were identical with those produced by the bean fungus, *Colletotrichum truncatum* (Schw.), n. comb.

Only Certain Strains of Tobacco Mosaic Cause Mosaic Burn.—W. D. VALLEAU AND E. M. JOHNSON.

Attempts to produce mosaic burn experimentally, using known strains of the virus, demonstrated that certain strains are entirely incapable of causing the disease. Inoculations made from mosaic-burned plants in the field regularly produced burn in the field and sometimes in the greenhouse. Dried specimens, collected 8 years ago from burned plants, still retained the ability to cause burning. If the finger method is used, necrosis typical of mosaic burn develops at the points of inoculation with the burn virus, but not with the nonburning strains. The local spots are distinct from the type of spots that occur on inoculated leaves of *Nicotiana glutinosa*. Inoculation of White Burley tobacco with a mosaic-burn virus at topping time has a tendency to increase the value of the upper leaves, changing the burned leaves from red tips to a smoker grade. The burned-tip leaves have much the appearance of flyings.

Further Studies on the Effect of Ammonia Nitrogen on Growth of the Cotton-root-rot Fungus, Phymatotrichum omnivorum, in Field and Laboratory Experiments.—D. C. NEAL.

Experiments conducted at Greenville, Texas, for the past 4 years have shown that the cotton-root-rot fungus, *Phymatotrichum omnivorum*, is highly sensitive to ammonia and ammonium compounds. In previous nutrition studies, it was found that ammonia nitrogen, when used in Duggar's solution at concentrations to yield 12.4 or 6.2 grams, respectively, of N per litre, has a toxic effect on the fungus, whereas similar concentrations of nitrate nitrogen produce an abundant growth. Additional experiments conducted in 1934, but with the nitrogen content still further reduced (supplied to cultures to yield 2.8 grams per litre), have largely confirmed the results of previous studies. No growth of mycelium occurred after 15, 30, 45, and 60 days with ammonium phosphate or ammonium sulphate; scant growth appeared with ammonium nitrate and di-ammonium phosphate after 15 and 30 days; and moderate growth after 45 and 60 days. In the

nitrate-nitrogen group, the best growth occurred, as heretofore, when calcium nitrate was supplied as the N source.

Sclerotia and hyphal strands formed only in cultures supplied with calcium nitrate and di-ammonium phosphate.

Concentrations of 50 p.p.m. NH_3 killed the root-rot mycelium in sectional-agar blocks in 24 hours, and concentrations of 300 p.p.m. killed the sclerotia in 1 hour. The residual effects of field applications of ammonium compounds made in 1932 were still marked in 1934 in both plot and barrier treatments, and show that the use of ammonia as the N source for certain Texas blackland soils may be a means of avoiding serious losses from root rot.

Control of Diseases of Tomato Seedlings.—FRANK VAN HALTERN.

During 4 years' cooperative tomato seed treatment and seed-bed spraying tests with 4 northern experiment stations have revealed that copper-lime dust did not control Macrosporium blight, OXO Bordeaux gave good disease control, and homemade Bordeaux mixture gave best results. The latter, combined with seed treatment, controlled Macrosporium and bacterial spot while without seed treatment control was fair.

Heavy seed dusting with Semesan Jr. reduced the stand but controlled Macrosporium blight, Semesan failed to control, and a 5 minute 1–1000 mecuric chloride solution controlled Macrosporium blight and bacterial spot. Monohydrated copper sulphate dust caused severe injury in dry, sandy soils.

Macrosporium overwintered in the soil but in two tests bacterial-canker failed to overwinter. Bacterial-canker was held down to a small amount by a seed treatment and spray combination.

Recommendations suggest state certified seed, a 5-minute treatment in a 1–3000 mercuric chloride solution, crop rotation, and spraying with 2–3–50 Bordeaux mixture when the second rough leaf appears followed by 3–4–50 sprays at intervals not exceeding 2 weeks.

Do Necrotic Lesions Result in Localization of Tobacco-Mosaic Viruses in Nicotiana?—W. D. VALLEAU.

Studies originally designed to put into practice the control of tobacco mosaic through localization of the virus in necrotic spots have demonstrated that necrotic spotting around the points of entrance of the virus and failure to become systemic are not correlated in species of Nicotiana. Seven strains of mosaic have been found that cause local necrotic spotting in several varieties of *N. tabacum* but not in others. In each variety these viruses became systemic. The factor for necrotic spotting in *N. tabacum* is a Mendelian dominant. Plants of *Nicotiana acuminata, N. langsdorffii, N. sanderae* and *N. rustica* inoculated each with one of several strains of tobacco mosaic developed necrotic spotting, but this did not localize the virus. In each species the virus spreads slowly from the leaf into the stalk, sometimes becoming systemic in *N. langsdorffii* and *N. rustica,* partially systemic in *N. sanderae,* and causing stalk necrosis and streak in *N. acuminata.* In *N. tabacum* var. *Ambalema,* necrotic spot-producing viruses failed to become systemic, probably because of resistance in this variety rather than because of necrotic spotting. With the possible exception, of Ambalema, necrotic spotting appears to be associated with a high degree of sensitivity to the virus resulting in extensive necrosis as the virus spreads.

The Etiology of Damping Off of Cotton Seedlings.—C. H. ARNDT.

Glomerella gossypii caused heavy losses of cotton seedlings in South Carolina in 1934. Greenhouse studies have shown that this fungus may cause damping off at soil temperatures

as high as 30° C. Other parasites that have been isolated from diseased hypocotyls of cotton in South Carolina are: 1. Nematodes. Several species of free living nematodes frequently invade normal hypocotyls. They apparently do not produce damping off when not associated with bacteria and fungi. 2. Fusarium spp. Fifteen to 75 per cent of the isolates from diseased hypocotyls have been species of Fusarium. Those which have been found sufficiently virulent to cause damping off have not been isolated frequently enough to indicate that they are a significant cause of losses of stand. 3. *Pythium ultimum* was found to be a frequent cause of poor germination and loss of stand in one field. 4. Bacteria. All attempts to produce typical damping off with the bacteria isolated from diseased hypocotyls have been unsuccessful. 5. Rhizoctonia. This fungus has been found on less than 10 per cent of the diseased hypocotyls examined during the past 10 years. These results do not support the generally accepted belief that Rhizoctonia is the usual cause of the damping off of cotton seedlings.

Control of Potash Hunger and Fusarium Wilt in Cotton.—V. H. YOUNG, J. O. WARE, and O. A. POPE.

Studies with wilt resistant varieties of cotton in western Arkansas in 1934 gave results comparable with those secured during 1927–1934 in eastern Arkansas. Of the 18 varieties studied, five showed from 4 to 8 per cent of cotton wilt compared to 29 to 44 per cent for the 5 most susceptible varieties. Highly resistant varieties were Clevewilt 33–6, Dixie 14, Rhyne's Clevewilt 33–10, Coker's Clevewilt 3 and Rhyne's Clevewilt. Highly susceptible varieties were Startex, Acala 1114, Super Cleveland, Trice 304, and Half and Half In eastern Arkansas where 36 varieties were studied, the incidence of cotton wilt ranged from about 1 per cent in Rhyne's Clevewilt to 49 per cent in Half and Half. In most cases a low incidence of cotton wilt was correlated with high yields and those varieties most severely attacked were invariably in the low-yielding group.

Five years' work indicates that the control of potash hunger or "rust" through the use of potash-containing fertilizers results, under central and eastern Arkansas conditions, in a marked decrease in the severity of cotton-wilt attacks. In 1933 and 1934 the use of a resistant variety of cotton (Rowden 2088) and potash fertilization gave the best control of "rust" and cotton wilt. On potash-depleted soils in Arkansas the control of potash hunger and wilt through potash fertilization and a suitable wilt-resistant variety of cotton proved to be the best control for these troubles available.

Seeds of Watermelons and Okra as Possible Carriers of Fusarium Wilt.—By J. J. TAUBENHAUS.

Extensive cultures were made of the roots, stems, pods, or rinds, and seed of watermelon and okra plants infected, respectively, by *Fusarium niveum* and *F. vasinfectum*. *F. niveum* was recovered from all parts of the roots, stems, peduncles, rind, and from only a very small percentage of the interior of the seed of fruit from infected watermelon plants cultured. Similar results were obtained with okra, namely, *F. vasinfectum* was recovered from all parts of the roots, stems, pod tissue, and from only a very small percentage of the interior of the seed from the pods of such plants. These results indicate that the seeds from either infected watermelons or okra may act as internal carriers of fusarium wilt.

On a Black Crown Rot of Greenhouse Snapdragons Caused by Myrothecium roridum Tode.—By J. J. TAUBENHAUS.

During the fall and winter of 1933 and 1934, snapdragons in a commercial greenhouse at Waco, Texas, were dying from a crown rot that killed over 90 per cent of the plants.

The affected crowns are at first water-soaked, then shrink, dry, and become covered with a thin white mycelium and numerous black sporodochia; finally, the epidermis and cambium crack open and become shredded. A fungus, *Myrothecium roridum*, was recovered from the infected tissue. The organism was successfully used to inoculate normal plants in which the disease with its typical symptoms subsequently developed.

Mode of Action of Bordeaux on Mycosphaerella fragariae.—A. G. PLAKIDAS.

Previous studies had shown: (1) That infection with the leaf-spot fungus obtains primarily through the dorsal surface of the leaf; and (2) that the disease can be controlled effectively by spraying with Bordeaux, even though very little of the spray reach the dorsal side of leaf. The present study has shown that Bordeaux is not protective in the sense that it protects the sound sprayed leaves by killing the sports that fall on them, thus preventing infection, but rather that Bordeaux affects the inoculum at its source. The spray does not penetrate to kill the mycelium in the tissue, but it greatly inhibits spore production on the sprayed spotted leaves. Comparative spore counts from sprayed and nonsprayed leaves showed that between 6 and 10 times as many spores were produced on the latter as on the former. Bordeaux is lethal to the nongerminated spores, even in relatively high dilutions. One hour's exposure to 1–21 dilution of a 4–4–50 Bordeaux mixture resulted in 100 per cent killing of the spores.

A Resumé of Cottonseed Treatments in South Carolina.—C. H. ARNDT.

A resumé was given of the effect of cottonseed treatments with dusts of the type of Ceresan on germination and yields in South Carolina since 1929. The data as presented were a composite of the results of W. D. Moore, 1929–30; E. E. Hall at the Pee Dee Experiment Station 1930–34; and for the Clemson College Experiment Station 1931–34. Treating natural seed increased the average stand of seedlings 18 per cent and increased the average yield 5.7 per cent. Acid delinting of the seed increased the average stand 50 per cent and the average yield 8.5 per cent as compared to natural, linted seed. Treating acid-delinted seed increased the average stand 25 per cent as compared to nontreated delinted seed, but did not increase the average yield. Machine-delinted seed gave an average increase of 80 per cent in stand, and increased the average yield 1 per cent in tests over a 3-year period as compared to natural seed. Treating machine-delinted seed gave an additional average increase in stands of 35 per cent, and 5 per cent in the average yields. Dr. Moore's results show significant reductions in the amount of angular leaf spot when treated seed was used.

The Reaction of Several Isolations of the Cotton-Wilt Fungus to Toxic Dyes.—GEO. M. ARMSTRONG AND C. N. CLAYTON.

One of the best of wilt-resistant cottons at Hartsville, South Carolina, wilted badly at Manning, S. C., in 1931. Cut stems of this variety from Manning showed a blackened ring in the center, while most of those plants from Hartsville showed blackening chiefly in the outer edge of the fibrovascular bundles. White and lilac to rose purple cultures were obtained. An attempt to differentiate between probable strains of the fungus, *Fusarium vasinfectum*, by use of 4 cotton varieties grown in the greenhouse was inconclusive. Coons and Strong's method of diagnosing species of Fusarium by means of growth-inhibiting substances in a synthetic culture, using malachite green and crystal violet, was then tried. Three dye tests have been made, with 8 cultures from different sources, noting color, size of colony, tolerance for toxic dye, and general growth characters. There were changes in color production by the same strain from test to test, and also changes in tolerance for the toxic dyes. The changes in color, growth, and tolerance for toxic dyes are in keeping

with the changes in various Fusarium species, as reported by Leonian and others. Our results with *F. vasinfectum* also are similar to those of Sleeth with *F. niveum*, in that a classification of "strains" made at one time will not be the same as that made at another time. A field test with these isolates is desirable, but there has been no opportunity to test them.

Studies on Downy Mildew of Tobacco.—G. M. ARMSTRONG AND C. B. SUMNER.

Three methods of control of tobacco downy mildew have been tried: sprays, high temperature, and forced air ventilation. Colloidal copper and Cal-Mo-Sul sprays saved many small plants. If the disease was delayed until plants were almost ready to set in the field, the sprays were of no value since practically all plants, regardless of treatment, became infected and few plants were killed even without the sprays. Cal-Mo-Sul, in 1934, gave a harmful residual effect in the soil. Five experiments on control by high temperatures, conducted in well-built beds, covered either by glass, Cel-O-Glass, Vio-Ray, Flex-O-Glass, or cloth, and heated by either electric light bulbs, G. E. heating cables, kerosene lanterns, or hot water in pipes heated by a kerosene heater, have shown minimum temperatures of 34° C. and 31° C. controlled the disease, but a minimum temperature of 29° C. or lower gave unsatisfactory control. Glass sash was superior to the other cover materials tried. Kerosene lanterns proved impracticable. In two instances, air heated by charcoal fires was conducted through flues either underground or along the inside of the beds. High temperatures were not maintained throughout the night. Outside air forced over a heating device into the bed protected fairly large plants but smaller ones near the soil were killed.

Dissemination of the Bacterial Leaf Spot Organism.—F. M. ROLFS.

The causal organism, *Bacterium malvacearum*, is carried from crop to crop on the seed. Seed is the source of both primary and secondary infection. There are two means of internal seed infection: Direct, by an invasion while the boll is quite young; indirect, by contaminated water through a break in outer seed coat of mature seed at the micropyle.

Water is the most important agent of dissemination. The bacteria were carried 980 feet in rill water. The green leaf bacteria have an independent movement in still water of 12 inches or more. 60 hours is the approximate age limit of the free bacteria in distilled water. Soluble soil salts in contaminated pool water shorten the life of the bacteria. Hot, dry weather tends to eliminate the more exposed parenchyma infections. The leaf spots become less angular in form, and the vascular invasion develops more prominently. The massing of the bacteria in veins and midrib hastens the shedding of the leaves, and affords better protection for the parasite from adverse weather conditions.

Wind is the most active agent of dissemination. A single whirlwind scattered the infected dry leaf material over an area of 100 acres in 20 minutes.

Breeding Peanuts for Disease Resistance.—B. B. HIGGINS.

Peanut breeding by the writer was started in 1931, with the objective of obtaining disease-resistant varieties better suited to southern Georgia conditions than those now grown. Approximately 200 crosses have been made, including various combinations between 15 varieties, each of which possessed some desirable characteristics. Complete records were kept of the principal characteristics of each progeny plant—about 200 first. Preliminary study of the data collected indicated that in most of the characters under consideration the first-generation plants are intermediate between the 2 parent varieties. In the second generation they break up in approximate Mendelian ratios. Resistance to the 2 leaf spots (*Cercospora personata* and *C. arachadis*) is apparently inherited independently. Selections, very resistant to one, are often extremely susceptible to the other.

No exact data have been obtainable on the inheritance of resistance to *Sclerotium rolfsii;* but we find some hybrid strains extremely susceptible and others quite resistant.

Results from Ten Years' Work on Cotton Seed Treatment.—B. B. HIGGINS.

In a more than 10-year field study of the efficiency of various disinfectant treatments for cotton seed some have given perfect control of angular leaf spot and considerable reduction of seedling anthracnose. Our records show small but consistent yield increases from the better dust treatments, even when planted in plats alternated with plats or rows planted with nontreated seed. Apparently, this increase was correlated with the better stand obtained with treated seed. In isolated walled beds constructed in a field where cotton had not been grown for 25 years, treated seed gave 1½ to 4 times the yield obtained from the check bed with an equal number of plants from nontreated seed, the first 3 years. The fourth year 100 lbs. of cotton seed was worked into the soil of each bed during the winter in order to get the soil evenly infested with the soil organisms usually carried on seed, especially *Fusarium moniliforme.* During the following 3 years the yields from the check bed have approximately equaled those from beds planted with treated seed.

The results indicate that disinfection of cotton seed may have considerable value for seed of low germination, such as that saved during a rainy season, and especially valuable when planting on land not devoted to cotton for several years.

Report of One Year's Test of Copper Fungicides and Other New Materials as Peach Sprays.—L. E. MILES.

Two applications of each of the following sprays were made on plats consisting of 5 trees for each of the treatments: (1) Self-boiled lime sulphur, 8–8–50; (2) wettable sulphur, 4 pounds, water, 50 gallons; (3) lime-sulphur concentrate, 1 gallon, Precipto, 1 quart, lime, 4 pounds, water, 50 gallons; (4) copper silicate, 2 pounds, Bentonite, 2 pounds, lime, 4 pounds, water, 50 gallons; (5) copper oxide, 2 pounds, Bentonite, 2 pounds, lime, 4 pounds, water, 50 gallons. Sprays were applied May 11 and May 30.

Brown-rot control was almost complete with all treatments. In no case was scab serious enough to affect the keeping quality of the fruit or to influence its market value except on a very discriminating market. The copper silicate treatment showed the greatest amount of both scab and spray burn with the copper oxide treatment next. The lime-sulphur concentrate and Precipto spray caused some spray injury both on foliage and fruit, but it was of negligible importance. Self-boiled lime sulphur gave best results in all cases.

Tobacco Bed Soil Disinfection.—J. O. ANDES.

Tests conducted over three reasons for tobacco bed disinfection indicate that a 3 per cent ammonium thiocyanate solution applied at the rate of 2 quarts per square foot, apparently disinfects the soil satisfactorily, judging by the killing of common weed seeds. The application should be made six weeks before seeding in order for it to disintegrate and become nontoxic to the tobacco seed.

The Verticillium Wilt Disease of Cotton.—L. E. MILES.

This disease was first reported in Mississippi in the fall of 1930. It has been found in 9 counties in the State and reported from Tennessee, Arkansas, California, Oklahoma, on 2 plants in Virginia, and from Greece. In 1933 field inoculations were made in July with strains of Verticillium (1) from cotton at Stoneville, Mississippi, (2) from cotton in California, and (3) from Irish potatoes in St. Catherines, Canada. The strain from Mississippi produced evident infection within 10 days. In 2 weeks 100 per cent of the

inoculated plants were severely infected and wilted. The strain from Canada produced no infection. That from California caused infection on 16 per cent of the inoculated plants, though the symptoms never were so pronounced nor the injury so great as from the Mississippi strain. The California strain caused no mottling or blotching, though those symptoms were pronounced on plants infected with the Mississippi strain. Similar inoculations identically made during hot, dry weather of August gave no infection with either strain. Potted greenhouse tomatoes were inoculated with the same strains in the spring of 1933. The Canadian strain caused much severer and earlier symptoms than did either of the others. Very little difference was observed in the effect produced by the Mississippi and California strains. Inoculations on eggplants, snapdragons, beets, and California poppy showed the Mississippi and California strains much more virulent than the fungus from Canada. The latter caused no infection on California poppy. The California strain also differs from the others in morphology of the fungus in culture. It produces no microsclerotia, whereas the others do.

Angular Leaf Spot of Cotton in Mississippi in 1934.—L. E. MILES.

A serious epiphytotic of angular leaf spot appeared on cotton in the Yazoo-Mississippi Delta section of Mississippi following a tropical storm that swept that area on June 16 and 17. The trouble was prevalent also in some other parts of the State, but nowhere to the same extent as in the above area. The heavily infested district comprises the 13 northwestern counties, in which the greater part of the cotton of the State is grown. Spread of the trouble was directly attributable to wind-blown rain. A survey made in early July showed very severe defoliation and shedding of squares and young bolls. In many fields 100 per cent of the plants were affected and 60 per cent of the foliage was shed, the fallen leaves completely covering the ground between the rows. In such fields, where dry weather throughout the season followed, secondary infection was limited but the plants and bolls remained small and developed abnormally. Where rains occurred at intervals during the season secondary spread to leaves and young bolls was heavy. Counts during the fall and winter showed abnormally low germination throughout the entire area in most cases, probably directly due to angular leaf spot subsequent to the heavy defoliation and, especially, in the drier areas, to the failure of the bolls to develop to full size and maturity.

With the opening of the bolls, heavy seed infection occurred from bacteria dripping in rain and dew from boll and foliage lesions onto the exposed lint. Samples of seed taken at random from the infested district have produced a high percentage of infected seedlings.

Efforts have been made to have as much seed acid-delinted or treated with 2 per cent Ceresan dust as possible to secure protection of the new crop.

Anthracnose and Wilt of Strawberry Caused by Colletotrichum fragariae.—A. N. BROOKS.

Losses from anthracnose or runner spot and wilt have been very high during certain summers in the central Florida strawberry area. Girdling of runners prevents development of new plants, and rotting of rhizomes results in wilting and death of plants that have become established. The disease symptoms are (1) typical anthracnose-like spots on runners and petioles and (2) reddish brown, dry rot of rhizomes, which causes wilting and death of plants. The causal organism is *Colletotrichum fragariae.* Environmental factors that favor rapid development of the disease are temperatures above 75° F. and high atmospheric humidity and soil moisture. Various spray mixtures and dusts have been used during the past 2 summers in experiments attempting to control the disease. Under periods of frequent rainfall all of the materials have failed to control, but, under

somewhat drier conditions, a pine oil containing 15 per cent copper resinate gave the highest percentage of control.

Wilt-resistant Cottons Adapted to the Gulf Coastal Plains.—D. C. NEAL.

In experiments conducted in cooperation with the South Mississippi Branch Experiment Station, at Poplarville, from 1932 to 1934, inclusive, the most adaptable and wilt-resistant cottons for that area have been selections of Dixie-Triumph, Miller, and D. & P. L. With these cottons, yields in excess of a bale per acre have been made under conditions of severe wilt infestation. Also, several selections have yielded a good quality of fiber of the desired length, ranging, in the case of the Dixie-Triumph, from 1 to 1 1/16 inches and from 15/16 to 1 inch in the case of the Miller. These cottons also are vigorous and apparently more resistant to leaf-spots than other varieties.

Other cottons that also have exhibited some degree of wilt-resistance in these tests, but are less adapted to the above area, are selections of Acala, Cook, Toole, Nucala, Clevewilt, Super Seven, Lightning Express, and Mexican Big Boll.

The Direct Effect of Bordeaux Mixture on Early Cucumber Production.—GEORGE F. WEBER.

Studies during the past season at Gainesville, Florida, have indicated that cucumber plants infected with downy mildew show increase in yield from the application of 2–4–50 Bordeaux mixture only when the picking season continues for more than 30 days.

Flower and fruit production, determined by daily counts throughout the season on 66 sprayed plants and 66 nonsprayed plants, showed that the nonsprayed ones produced more staminate and pistillate flowers and set more fruit each week for 3 consecutive weeks from the beginning of the season than did the sprayed plants. During the fourth week and thereafter, the sprayed plants exceeded the nonsprayed plants in flower and fruit production, because the latter were more than half defoliated by the disease at the end of the third week.

Sclerotinia Rot of Irish Potatoes in Florida.—A. H. EDDINS.

Sclerotinia rot of Irish potatoes caused by *Sclerotinia sclerotiorum* (Lib.) Mass., occurs generally throughout the Hastings potato area where, in 1933 and 1934, it was epidemic in a few fields. The fungus attacks the main stem, branches, and leaves of the potato plant. The tubers are not invaded but their growth is checked when the top of the plant is damaged by the disease. Epidemics of the disease occur on low ground during cloudy, foggy, and rainy weather. In 1934 70 per cent of the plants growing in the low areas in one field were killed by sclerotinia rot. Maximum yield reductions result when the main stems of the plants are invaded by the fungus 30 or more days prior to digging.

A Root Rot of Strawberry Caused by a Species of Diplodia.—R. E. NOLAN.

A quick-killing root rot of strawberry has been found to be caused by a species of Diplodia. The organism is readily isolated and inoculation experiments have proved it to be the cause of the disease. The disease is first noticed as a small dark brown spot on the roots, which quickly rots the entire root. Sometimes the affected root breaks off near the origin of infection, but the disease continues up to the plant. Inoculated plants are killed in from 6 to 10 days during hot weather. Soil-temperature experiments show that it is most destructive when the temperature is above 80° F. The organism belongs to the "natalensis" group of the genus Diplodia. Comparison with several other species of this group shows that it has characteristics common to some of them and varies in other characters. Cross-inoculation experiments with the several species also gave variable re-

sults. It is more active than *D. frumenti* in infecting corn, while, of the species used in comparison, *D. natalensis* attacked the strawberry more readily than any of the others.

Soil-temperature Studies on Florida Cigar-wrapper Tobacco.—R. R. KINCAID AND L. O. GRATZ.

Black shank (*Phytophthora parasitica* var. *nicotianae* Tucker) is a most important disease of Florida cigar-wrapper tobacco. Extensive experiments under controlled conditions of soil temperature and known soil moisture were conducted for the purpose of establishing the cardinal soil temperatures for the growth of transplanted tobacco seedlings, as well as for the development of the disease. Resistant No. 301 and susceptible Round Tip tobacco were used in these experiments. The cardinal temperatures for the growth of transplanted seedlings were found to be 9°, 28°, and 40° C. The maximum for the development of black shank varied from 16° or lower for newly transplanted seedlings to about 24° for plants inoculated several weeks after transplanting; the optimum and the maximum were about 28° and 34°, respectively. Some field observations indicated that soil temperature is an important factor in the development of black shank in the field.

The Effect of Certain Environmental Factors on the Germination of Florida Cigar-wrapper Tobacco Seeds.—R. R. KINCAID.

The cardinal temperatures for germination are approximately 10°, 24°, and 34° C. A daily alternation between 20° for 8 hours and 30° for 16 hours produces more rapid germination than a constant temperature of 24°. Oxygen concentrations of 60 per cent and higher are unfavorable for germination. The apparent isoelectric point of the seeds is about pH 6.8, as shown by bimodal curves of germination and water absorption and by ion-adsorption experiments. All of the 19 samples of seeds tested required light for germination at constant temperature. The amount of visible radiation required is of the order of 10–11 gram-calorie per seed. The seeds must be imbibed with water at the time they are exposed to light, but the effect of light is not reversed by subsequent drying. The effect of light on imbibed seeds is independent of the temperature of the seeds at the time of exposure. The requirement of light for germination may be offset by a sharp daily alternation of temperature during incubation.

Field Studies of Fusarium Wilt of Cotton.—C. D. SHERBAKOFF AND G. M. STONE.

A study of the development of fusarium wilt of cotton as affected by soil conditions was begun in western Tennessee in the summer of 1934. Data thus far obtained indicate that there is a correlation—most likely an indirect one—between the topography, the pH value, and the amount of organic matter in the soil on the one hand, and development of the wilt on the other. The greater number of wilted plants were found on the higher elevations, where the soil was less acid and its organic content low.

Effect of Crown Gall, Hairy Root, and Woolly Aphids on Apple Trees in the Orchard.—C. D. SHERBAKOFF AND J. A. MCCLINTOCK.

A test orchard of 1200 apple trees was set out at Jackson, Tennessee, in 1923, to determine what effect the presence of crown gall, hairy root, and woolly-aphid knot on nursery stock at planting time have upon the trees in the orchard. The nursery trees were grouped according to their condition and were set out without being fumigated or disinfected. Notes taken 1, 3, and 6 seasons later show apparently significant differences among the groups. Compared with trees, apparently healthy at planting time, there were 4 or more times as many dead, or dead and dying, among those with large crown galls or

extensive development of woolly-aphid knots, and 2 or more times as many among those with hairy roots, small crown galls, or slight development of woolly-aphid knots.

Control of Sweet-potato Stem Rot in West Tennessee.—G. M. STONE.

A survey was made in 1934 of the factors contributing to sweet-potato stem-rot infection, in typical fields of Weakley County—the leading sweet-potato county of Tennessee. During the same season and under the same field conditions, the crop on 2 farms was grown under the supervision of the writer, where certain necessary methods of stem-rot control were employed. The survey and test show, (1) that the best control of stem rot is obtained where new fields were set with Bordeaux-dipped slips produced in new beds, from certified, properly graded, and disinfected seed; and (2) that the factors contributing most to infection are, in the order named, old beds, contaminated fields, contaminated and ungraded seed.

Lespedeza sericea Stem Blight.—J. K. UNDERWOOD.

In 1933 and 1934 considerable loss from "stem blight" was noted on *Lespedeza sericea* in eastern Tennessee. The affected plants show a general yellowing, and the bark becomes shrunken and discolored. A Phoma-like organism has been found constantly associated with the disease and is suspected of being the cause of it. Positive infections were obtained when inoculations of disease-free seedlings were made with the fungus. The differences in the symptoms, however, indicate the possibility that the disease is more complex, or of a different causation, than one may conclude from the findings.

Black Root Rot of Apple.—J. O. ANDES.

Black root rot of apple has been found scattered throughout Tennessee. Only *Xylaria mali* Fromme has been isolated from or found in connection with the diseased roots. Preliminary tests and observations suggest that development of the disease can be arrested by sterilizing the immediate area of diseased trees. A treatment with a combination of steam and formaldehyde appears to be the most economical method tried for this purpose. Observations indicate a possibility of nursery trees introducing the disease.

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RUST RESISTANCE IN ANTIRRHINUM[1]

E. B. MAINS

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Snapdragon rust, *Puccinia Antirrhini,* was described by Dietel and Holway (6) from specimens of rusted *Antirrhinum majus* collected by W. C. Blasdale at Berkeley, California, in 1895. Blasdale (3) in 1903 stated that it was common in the region around San Francisco Bay but that he had no knowledge of its occurrence elsewhere in the State. At that time the rust appeared every year in his garden with such severity that the infected plants of snapdragon were killed shortly after reaching the flowering stage. Peltier (15) reported the rust as severe in the vicinity of Chicago in 1913. By 1921 (7, 15), it was widely distributed throughout temperate North America. It now is known (2) in 24 States of the United States, 5 Provinces of Canada, and in Bermuda. Until recently (1933) it has been unknown in Europe. It is now reported (9) in England, Belgium and Germany.

Blasdale (4) found that, in addition to *Antirrhinum majus,* the rust occurred on *A. Nuttallianum* and *A. virga* in California. He reports success in infecting *A. vagans, A. assurgens, A. nudicum, Linaria amethystina, L. delphinifolia, L. bipartita, L. melanthera,* and *L. macrocanna.* Arthur (2) reports the rust on *A. Nuttallianum* and *A. virga* in California. He also includes rust on *Cordylanthus* (*Adenostegia*) *filifolius, C. pilosus* and *C. rigidus.* Because *Antirrhinum majus* is native of the Mediterranean region, where the rust is unknown, and became rusted only after snapdragons were introduced into California, it is evident, as Blasdale (3) has suggested, that the rust was indigenous on Californian species of Antirrhinum and found snapdragons a congenial host where the latter were introduced.

Studies concerning the host range of the rust have been reported also by Peltier (15) and Emsweller and Jones (8). Peltier states that he inoculated several species of Antirrhinum (not specified), a large number of commercial varieties of *A. majus, Linaria vulgaris* and a number of species and commercial varieties of Linaria (not specified). All the varieties of *A.*

[1] Papers from the Department of Botany and Herbarium of the University of Michigan No. 520. These investigations were started while the writer was a member of the Department of Botany, Purdue Agricultural Experiment Station, and have been continued at the University of Michigan.

majus were severely infected. A slight infection was obtained on *A. maurandioides* Gray. "No infection was obtained on *L. vulgaris* or on any other species or commercial varieties of Linaria. This was true also of Antirrhinum species with the exception of *A. majus*. . . ." Emsweller and Jones studied a number of species of Antirrhinum collected by E. Baur in Spain. A collection of *A. Ibanjezii* and a collection of *A. siculum* were resistant. A collection of *A. tortuosum* was susceptible. A strain of *A. glutinosum* and of *A. molle* proved to be resistant, while a second strain of each species was susceptible. A collection of *A. hispanicum* contained both resistant and susceptible plants.

Puccinia Antirrhini is apparently heteroecious. Mains (12) and Hockey (10) have germinated the teliospores. Snapdragons were inoculated without obtaining infection. The hosts for the aecial stage are unknown.

In the United States the rust has become an important factor in the culture of snapdragons. For a time it was specially serious in greenhouses where snapdragons were propagated by cuttings. Peltier (15) showed that the rust is not seed-carried. With the abandonment of propagation by cuttings and the adoption of such practises as subirrigation, regulation of temperature, etc. (7, 15), the rust is less serious in the greenhouse.

It still remains more or less important in the garden. When it does not result in the death of infected plants, it renders them unsightly, naturally a very undesirable condition for an ornamental. Also, in California, the rust often has important economic results, since most of the seed used in North America is grown there. Thus, in 1921, D. G. Milbrath wrote that the damage was the most that he had ever observed. One large seed grower lost his entire crop, valued at $10,000, and in other fields the crop was only 25 per cent of the normal.

Sulphur compounds, specially sulphur dusts, have been used successfully to greatly reduce the development of the rust (5, 7, 13). Butler (5) found that successful control varied from year to year, apparently correlated with temperature. Rotation of crops, early planting, withholding of water, have been employed in the seed-production districts of California (8). Although reducing the damage to some extent, none of these methods has been entirely satisfactory.

It is obvious that highly resistant strains would be the most desirable solution of the difficulty. Peltier (15) tested a large number of commercial varieties, all proving to be susceptible. Doran (7) reports a study of 46 varieties. His results are difficult to interpret. Under the heading "Scale, relative numbers" he has assigned values, 0 to 100, to the various varieties. He states that, while the most resistant varieties were not absolutely resis-

tant, they were the nearest to it among snapdragons. It would appear, therefore, that the designation "0" was not used to indicate immunity.

Little or no evidence of resistance has been noted by other investigators. D. G. Milbrath has informed the writer that in 1926 he inspected a large number of fields of snapdragons in California without finding any individuals outstanding for resistance. Emsweller and Jones (8) state that in 1929 a search for resistant individuals in the fields of the large seed growers of California was unsuccessful, and Emsweller, in a letter to the writer, has stated that over a period of 15 years no degree of resistance had been noted in the California seed fields.

The writer's studies started in 1922 with 2 selections that showed some resistance. The progress attained in developing resistant strains was reported (14) at the Nashville meeting of The American Phytopathological Society, in December, 1927. As a result, requests for seed were received and in 1928 and succeeding years several lines were sent to various investigators. Selections from these have been used by Emsweller and Jones (8) and by White (16) for developing rust-resistant lines of commercial types. The following account summarizes the development of these strains and the results obtained in additional studies.

METHODS

The technic of inoculation has been described in detail elsewhere (11). In brief the method used in greenhouse studies was as follows. Abundant inoculum was maintained on susceptible plants. Such heavily rusted individuals were shaken over the plants to be inoculated, thus dusting the latter with urediospores. They were then atomized with water and covered with muslin which was wetted. If this be done in the afternoon the lower temperatures during the night will result in a high humidity and condensed water on the leaves. In addition to production of conditions of humidity conducive to germination, the lower night temperatures also favored germination. The results obtained from a study of the effect of temperature upon germination of the urediospores and upon infection are not entirely in accord with those reported by Doran (7). The average germinations obtained at various temperatures were as follows: trace at 0° C.; 74% at 7° C.; 94% at 10° C.; 77% at 15° C.; 48% at 19° C.; 25% at 24° C.; 9% at 26° C.; trace at 30° C. These results are supported by infections obtained by inoculating at various temperatures (Fig. 1). Doran (7) found that 10° C. was the optimum temperature for germination, but reports that a decrease or increase of 2° C. resulted in a decrease of 50% in germination.[2] Inoculated

[2] It should be noted that Doran gives a value of 100 for the germination at 10° C. (actual percentage not stated) and records relative values for the results obtained at other temperatures. The discrepancy may possibly be explained by a difference in germinability of the spores employed in the two studies.

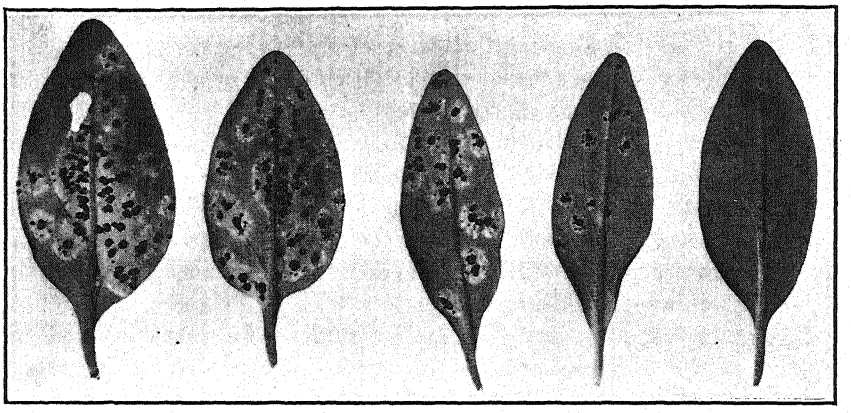


FIG. 1. Leaves selected to illustrate infection of susceptible snapdragons by *Puccinia Antirrhini* when inoculated at temperatures (left to right) of 10°, 15°, 19°, 22°, and 30° C.

plants were uncovered after 24–48 hours and infection appeared in 10 to 15 days, depending upon conditions of light and temperature.

In the field heavily infected plants were distributed at intervals throughout the plot. Infection spread from such sources. Susceptible strains were placed at intervals among the resistant selections and served for comparison.

In selecting for resistance, selected individuals were self-pollinated. Most commercial strains of snapdragons are self-fertile. If they are bagged to prevent pollination by insects, usually there is an abundant production of seed. However, difficulty was experienced under field conditions during the summer months when apparently high temperatures in the bags destroyed viability of pollen. As the study progressed it was found desirable to insure the application of pollen to the stigma. For this purpose wooden toothpicks with a broad flat end were found very useful. They can be obtained at little cost. Pollination is accomplished by drawing the pollen from the lower anthers over the stigma. After all the flowers of the spike are pollinated the toothpick is discarded.

RESULTS

This study originated from observations made in the writer's own garden. It was continued for a number of years in the Department of Botany of the Purdue University Agricultural Experiment Station, Lafayette, Indiana, as one of a number of miscellaneous investigations secondary to major projects. Consequently, the progress of the study was somewhat retarded because of limited time and facilities. During the first few years a study was made of varieties and selections of the snapdragon, *Antirrhinum majus*. Later, attention was turned to other species of Antirrhinum and to

the possibility of developing resistant snapdragons from crosses with such species.

COMMERCIAL STRAINS AND VARIETIES OF SNAPDRAGONS

In 1922 snapdragons of mixed colors in the writer's garden became heavily rusted. By autumn most of the plants were killed. However, two

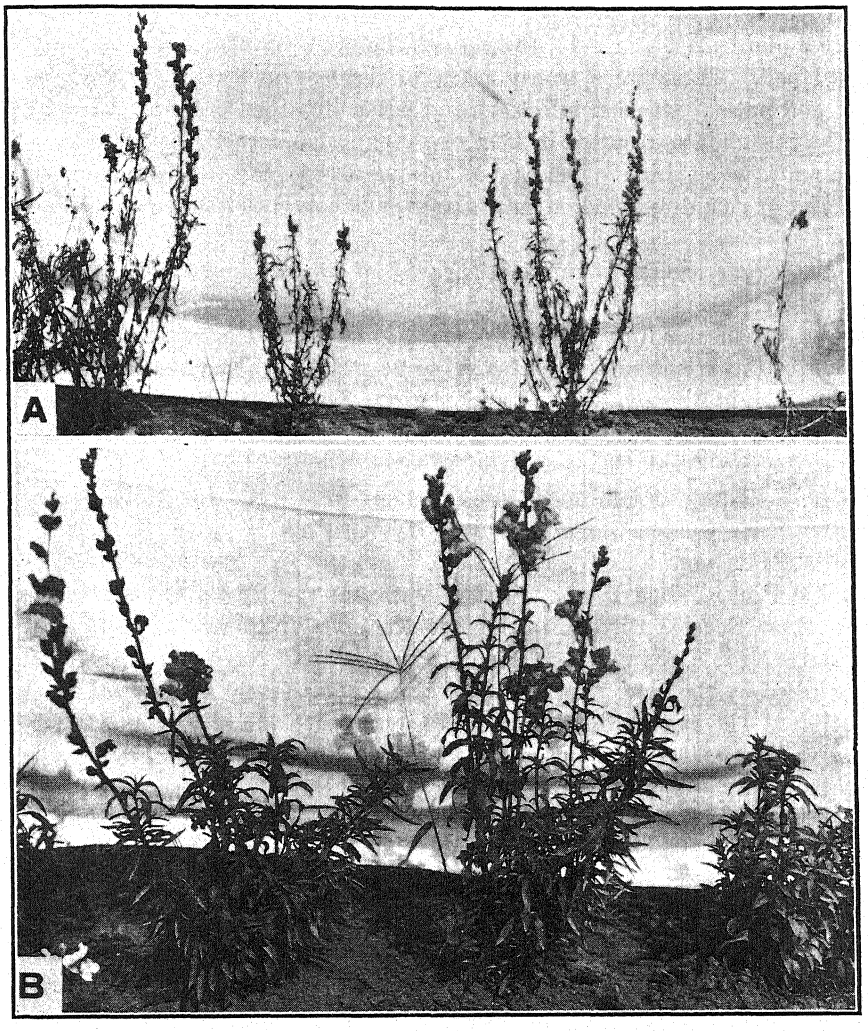


FIG. 2. A. Condition, due to rust, of plants of the variety Maralda in a field planting in September, 1927. B. Condition of resistant selections of the 7–13 strain of snapdragons in September, 1927, in a field planting where commercial strains were severely rusted.

plants survived, although moderately rusted. They were transplanted to the experiment station greenhouse and the flower spikes were bagged and self-pollinated. Young plants raised from seed thus produced were transplanted to the field the next summer and exposed to rust. Considerable variation occurred in the progenies. A number were killed by the rust. The majority were alive at the end of the season, although moderately rusted. A few were only 25 per cent rusted and the uredia were small. Among these were 2 plants, 2–1 and 7–13, from which the most promising lines were later developed. These were transplanted to the greenhouse where they were self-pollinated. Further selections and self-pollination, both in the field and in the greenhouse, resulted in 1928 in a number of lines of the 2–1 and 7–13 strains in which highly resistant plants occurred (Fig. 2). Only a slight amount of rust developed on such plants and the uredia produced were very small.

Each year several commercial varieties of snapdragons were included in the study. Among these were represented most of the colors and types offered by the leading seed companies of the United States. During the course of the investigation the following varieties have been studied: Amber Queen, Apple Blossom, Black Prince, Bronze Scarlet, the Bride, Buff Queen, Butterfly, Buxton, Canary Bird, Captivation, Cerise King, Chamois, Copper King, Cottage Maid, Crimson King, Delicata, Delila, Fascination, the Fawn, Feltham Beauty, Firebrand, Garnet, Giant White, Gloria, Golden Fairy, Golden Pink, Golden Queen, Gotelind, Hybrid Pink, Keystone, Los Angeles, Lovely, Maralda, Mont Blanc, Nelrose, Peerless Pink, Phelps White, Philadelphia Pink, Prima Donna, Purity, Queen of the North, Queen Victoria, Rose Dore, Scarlet, Silver Pink, Snowflake, and Wallflower. Most of these were susceptible, showing few or no differences. All the plants were eventually killed by the rust. In a few varieties an occasional plant was less susceptible. The rust developed more slowly on such plants and they usually survived until the end of the season. Such plants were selected in the varieties Butterfly, Canary Bird, Giant White, Gloria, Golden Queen, Peerless Pink, and Prima Donna. From most of these, after several years of inbreeding, lines somewhat less susceptible than the parent varieties were isolated. These, however, did not approach the 2–1 and 7–13 lines for resistance and, therefore, were not continued. However, one selection, G W D 1, from the variety Giant White, after several years of inbreeding, produced plants equalling those obtained in the 2–1 and 7–13 lines and produced a much better plant and flower type than those of the latter.

Table 1 gives the results obtained in the 1929–1930 greenhouse study of the most promising rust-resistant selections. Although no line had at that time been produced that was uniformly highly resistant, a number showed a marked improvement over commercial varieties as far as rust resistance

TABLE 1.—*Rust reaction in greenhouse of outstanding selections of snapdragons in 1929*

Selections or variety	Number of plants reacting[b]				
	0–t	1	2	3	4
2–1–1–1–G1–F1–G1		45	15		
2–1–1–1–G3–3	1	32		5	
2–1–1–2–G3–4	2	40	16		
2–1–1–3–G2–1	1	42	20	3	
7–13–8–1–G1–F1–G1		34	6	4	
7–13–8–1–G1–F2–3	3	33	8	2	
7–13–8–1–G6–F1–2		15	21	2	
7–13–8–1–G6–F2–F3		6	15	11	11
7–13–19–3–G3–3		48	15		
G W D1–G2–F1–1	1	15	4		
G W D1–G2–F3–3	1	25	5		
G W D1–G3–F1–2	5	35	3		
G W D1–B4–F2–3	8	38			
Nelrose[a]					55
Golden Fairy[a]					45

[a] Varieties from commercial seed included for comparison.

[b] 0–t, no uredia or few very small uredia; 1, few small uredia; 2, moderately rusted, uredia moderate size; 3, fairly heavily rusted, uredia moderate size; 4, severely rusted uredia large.

was concerned. As the inbreeding progressed, more or less sterility developed, especially in the 2–1 and 7–13 lines, which greatly retarded the inbreeding and selection of homozygous, highly resistant lines. This was especially unfortunate, since the number of individuals that could be studied was limited. Also, as a result of these restrictions and the necessity of concentrating efforts on the selection of desired types of resistance, it was not possible to give much consideration to other qualities. The most promising rust-resistant selections, especially in the 2–1 and 7–13 lines, unfortunately proved to be undesirable for commercial purposes. The G W D lines, although not all that could be desired, were the most promising both for rust resistance and other qualities. They produced moderately tall plants with white flowers of good size, fairly well-spaced on the spike.

Consequent upon publication of results reported in 1927 (14), requests for seed of resistant lines were received. It was evident that the utilization of the resistant selections would probably necessitate an extensive study of hybrids made with desirable types, a phase for which time and facilities were not available. It was decided, therefore, to distribute the most promising strains in order that they might be tested in other parts of the country and utilized, by those who so desired, to develop resistant strains of commercial

value. Subsequent efforts in the investigations reported here have been directed mostly in a continuation of the attempt to isolate highly resistant lines from as many sources as possible.

Seed of the most promising lines for resistance have consequently been sent from time to time to investigators in Arkansas, Bermuda, California, Massachusetts, Michigan, and Virginia. The reports received have been as follows: Seven strains were sent to D. G. Milbrath in 1927 and were tested by him at Sacramento, California. He reported that all of these rusted more slowly than the commercial strains with which they were compared. By the end of the season, 4 (G W D1–G2, G W D1–G3, 2–1–1–3–G2, 7–13–19–3–G3) had developed very little rust, averaging less than 30 sori per plant, 2 (7–13–F6–G1, 348–F2–F1) rusted to a greater extent but survived, and only one (2–1–1–3–G1) was seriously affected.

A number of lines were sent to J. B. Kendrick in 1928 and 1929 and were studied by him and by S. L. Emsweller at Colma, and Guadelupe, California. Their notes may be summarized as follows: At Colma the resistant strains were scattered through a commercial planting. All the strains selected for rust resistance were more resistant than the commercial types. Two were outstanding. Although in close association with commercial types that were dying from rust, most of the plants of 2–1–1–1–G3 were still in growing condition and 2 plants had but 2 per cent of the leaves rusted and these bore only 1–3 uredia. Strain 2–1–1–2–G3 was somewhat less resistant. The uredia were fewer and smaller than those on commercial strains. The other 5 strains were more severely infected, although less so than commercial strains.

At Guadelupe, in 1929, the rust was specially prevalent and the strains were subjected to a very severe test. In August only 8 out of 100 plants were dead in strain G W D1–G2–F1. The remainder averaged only 1 uredium per leaf. Only 10 out of 100 plants of G W D1–G2–F3 were dead and the remainder averaged only 2–5 uredia per leaf. Strain 7–13–19–3–G3 had 5 out of 100 plants dead, the remainder averaging 3–4 uredia per leaf. Strain 7–13–F6–G1–1 was similar. Of the other 6 strains, 4 had from 14 to 50 plants killed out of 100 and 2 had all the plants killed by the rust.

In 1930 additional strains were tested at Guadelupe. Too few plants were grown to justify a comparison between strains. However, 11 plants were noted that showed no rust.

The resistant plants in these tests did not meet the standards of the growers in regard to flower and plant types. However, Emsweller and Jones (8), starting with some of the most resistant survivals[3] and crossing

[3] In a letter Professor Emsweller informs me that their fully resistant strains originated from 2–1–1–1–G3 and 2–1–1–2–G3.

with commercial strains, have been successful in developing desirable commercial types possessing marked resistance.

In 1930 a number of strains were tested by Harold E. White at Chatham, Massachusetts. In August, when notes were taken, a number of the commercial varieties with which they were being compared had been completely killed by the rust. Thirty-seven out of 87 plants of the resistant strains showed little or no rust. The most outstanding strains were 2–1–1–1–G1–F1–G1 and 7–13–8–1–G6–F2–F1–G1 and selections from a cross between G W D1 and resistant selections of *Antirrhinum glutinosum,* which will be discussed later.

SPECIES OF ANTIRRHINUM AND RELATED GENERA

Another approach has been through wild species and strains of Antirrhinum. This phase of the investigation was started in 1926. At that time 8 species of Antirrhinum, 1 of Adenostegia and 6 of Linaria were studied. Additional species have been added from time to time as opportunity offered. Seed of a number of species was kindly supplied by E. Baur, F. Brieger, L. A. Waitsinger, and various Botanical Gardens. All the species of Linaria studied, *L. canadensis* Dum.-Cours, *L. Cymbalaria* Mill., *L. dalmatica* Mill., *L. pallida* Tenore, *L. purpurea* Mill., and *L. vulgaris* Mill., gave no evidence of infection. *Adenostegia pilosa* Greene (*Cordylanthus pilosus* A. Gray) also, was found to be highly resistant. It is to be noted that this is given as a host for *Puccinia Antirrhini* by Arthur (2). The results obtained with species of Antirrhinum are given in table 2.

In some instances seed germinated poorly, so that only a few plants were available for study. In such cases the results do not necessarily entirely represent the situation in the strain or the species. In these studies the species *A. asarina, A. Ibanjezii, A. maurandioides,* and *A. virga* were uniformly highly resistant and *A. Barrelieri, A. Coulterianum, A. glandulosum,* and *A. vexillo-calyculatum* were uniformly susceptible. The strains of *A. latifolium* and *A. Valentinum* studied contained a few individuals showing some resistance. *Antirrhinum Orontium,* for the most part, was moderately resistant. *Antirrhinum glutinosum, A. hispanicum, A. molle, A. Nuttallianum, A. sempervirens, A. siculum,* and *A. tortuosum* showed marked differences between or within strains of the species. Thus, in *A. glutinosum,* strain 200 contained highly resistant, moderately resistant, moderately susceptible, and very susceptible individuals, and strains B3 and B8 were uniformly highly resistant. The strains received from E. Baur as wild forms of *Antirrhinum majus* proved very interesting, since strain B6 was highly resistant and strains B7 and B8 contained both highly resistant and susceptible individuals. Emsweller and Jones (8) report resistance in *Antirrhinum glutinosum, A. hispanicum, A. Ibanjezii, A. molle,* and *A. siculum.*

TABLE 2.—*Reaction of species of Antirrhinum to rust*

Species	Accession No.	Number of plants reacting[a]				
		0–t	1	2	3	4
Antirrhinum asarina L.	239	19				
" "	1636	23				
" Barrelieri Bor.	1866					26
" Coulterianum Benth.	611				1	3
" glandulosum Lindl.	603				3	2
" "	612					28
" glutinosum Boiss. & Reut.	200	2	2	1	4	2
" " Lagerfels[b]	B3	8				
" " Orgiva[b]	B4	12				
" hispanicum Chav.	B10					11
" "	1868			1	3	8
" "	1638	2	1		14	3
" "	1634			1	1	13
" Ibanjezii Cartagena[b]	B5	12				
" latifolium Mill.	1128				2	3
" "	1869			1		21
" " Mentone[b]	B12			2	13	
" majus L. Lucena[b]	B6	16				
" " Loja[b]	B7	1				6
" " Cintra[b]	B8	6		15	10	12
" " Zaragoza[b]	B9	12	1	8		14
" maurandioides A. Gray	1121	16				
" molle L. Braganza[b]	B2				5	4
" " Monsech[b]	B1	12				
" Nuttallianum Benth.	605	11		16	6	7
" Orontium L.	1123	4	2	1		
" "	1872			33		
" sempervirens Lapeyr.	1120	8				
" "	1873			1	2	8
" sempervirens[c]	B13	18	26	6		13
" siculum Mill.[b]	B14	3	6	12	17	
" "	Br6				27	
" "	Br7				12	
" "		6	10		1	
" "	1635			27	11	
" "	1874			2		27
" tortuosum Bosc.[b]	B11	2		40		
" "	1637				17	18
" "	1875					31
" Valentinum[b]	B15			2	13	
" vexillo-calyculatum Kelloge...	606					17
" virga A. Gray	613	6				

[a] See Table 1 for explanation of reaction.

[b] Received under this name from E. Baur. See Zeitschrift für Induktive Adstammungs- und Vererbungslehre 63: 256–302. 1932.

[c] Received from E. Baur under designation A. meonanthum × Schw. B.

The marked resistance found in some of the species suggested the desirability of attempting crosses with commercial types of snapdragon. In 1927 resistant plants of *Antirrhinum glutinosum* 200 were crossed with the resistant strain, G W D1, of Giant White. The plants of the first generation

were all extremely resistant. The second generation produced 11 very susceptible, 40 moderately resistant, and 45 highly resistant plants. It was possible to continue only a limited number of the most promising resistant selections and these continued to give segregations into various susceptible and resistant types for the next 2 years. In 1932, 2 cases (9–1–3 and M12) of clear cut separation into an extremely resistant and a very susceptible group were obtained. The next year 5 lines proved to be homozygous for marked resistance. Usually there is no sign of infection when the plants are inoculated, although occasionally a few minute uredia may be produced. These lines in general, resemble the Giant White parent. The flowers are white but somewhat smaller than those of Giant White. They also show a tendency towards a narrow and somewhat pointed standard, characteristics derived from *Antirrhinum glutinosum*. While the writer considers that this has a certain grace as well as novelty, it is a departure from the standard and will probably not meet with the approval of the florist.

In 1932 several crosses were made with the most outstanding resistant segregates of 9–1–3 lines, using susceptible commercial types for the other parents. As stated above, the resistant lines were still segregating and, in the F_1, 2 of the crosses produced highly resistant and very susceptible segregates in ratios of 10:11 and 12:14. One produced only highly resistant plants. The F_2 from 3 resistant plants of the F_1 gave the following ratios, closely approximating 3 highly resistant and 1 very susceptible. The results obtained were as follows:

	Resistant	Susceptible	Deviation	Dev./P.E.
	109	40	2.75	.77
	83	36	6.25	1.96
	98	25	5.75	1.77
Total	290	101	3.25	.56

From this it is evident that the marked resistance of the 9–3–1 lines is a simple dominant factor. White (16) reports obtaining similar ratios in crosses using resistant plants from early selections in this material. He apparently has been successful in developing several highly resistant commercial types.

Among others a species labeled *Antirrhinum Ibanjezii* was received from E. Baur in 1932. As shown in table 2, this species proved to be highly rust resistant. It was crossed with a susceptible commercial pink snapdragon. The F_1 generation was uniformly (21 plants) highly resistant, similar to the *A. Ibanjezii* parent. The F_2 segregated in a ratio of approximately

3:1, giving 113 extremely resistant and 32 very susceptible individuals, a deviation of 4.25 and Dev./P.E. of 1.20. Here, also, resistance is apparently due to a simple dominant factor.

DISCUSSION

Puccinia Antirrhini, although first collected on snapdragon, *Antirrhinum majus,* and described from that species, apparently was originally endemic to the Pacific Coast of North America on various species of Antirrhinum. Of the 12 species listed by Jepson for California, 5 have been studied in these investigations. *Antirrhinum maurandioides* and *A. virga* proved to be highly resistant. *A. Nuttallianum* contained highly resistant, moderately resistant, and susceptible individuals. *Antirrhinum glandulosum, A. Coulterianum,* and *A. vagans* (*A. vexillo-calyculatum* according to Munz) were susceptible. Arthur lists *A. Nuttallianum* and *A. virga* as hosts for the rust in California. *Antirrhinum majus* is native to the Mediterranean region where the rust is unknown. The marked susceptibility and the rarity of resistance in *Antirrhinum majus* also might be considered as further evidence, since previous to introduction into California there would have been no opportunity for selection of resistant strains. However, this alone would not be sufficient, since it is interesting to note that other exotic species, such as *Antirrhinum glutinosum, A. hispanicum, A. molle,* and *A. siculum,* show fully as much or more resistance than the California species of Antirrhinum.

In these studies only species of Antirrhinum have been successfully infected. All the species of Linaria and Adenostegia investigated have proved resistant. The resistance of *A. pilosa* raises some interesting questions. Based on the telia collected on *A. pilosa* (*Cordylanthus pilosus*) Arthur (1) described the species *Puccinia Adenostegiae.* Later, Blasdale (4) proposed the species *Puccinia Cordylanthi* for rust collected on *Cordylanthus filifolius* (*Adenostegia filifolia*). Arthur (2) considers these as synonymous with *Puccinia Antirrhini,* thus adding *A. filifolia, A. pilosa,* and *A. rigida* as hosts for the species. The results reported here suggest that the rust on species of Adenostegia may be distinct, at least so far as its host specialization is concerned. However, since only one collection of *Adenostegia pilosa* was studied, it may be that a resistant strain of the species was employed. Further study with other collections of the species of Adenostegia and also with rust from Adenostegia on Antirrhinum is necessary.

The observations and studies of most investigators have indicated that the species *Antirrhinum majus,* as represented by cultivated varieties, is very susceptible. Only Doran (7) has reported any pronounced differences among such varieties. His data are difficult to interpret. Under the heading "Scale, relative numbers," he lists with the value 0 such varieties as

Queen of the North, Giant White, Queen Victoria, Mont Blanc, Rose Dore, and Firebrand, all of which, in the writer's studies, have been susceptible, under favorable conditions for rust development, being entirely or mostly killed. Doran (p. 62) states that "these varieties, while not absolutely resistant, are the nearest approach to it among snapdragons" and it would appear that he has employed a classification in which 0 is used as a designation for the least susceptible of the varieties studied by him.

In these investigations no pronounced differences have been noted between cultivated varieties when considered as a whole. Individual differences, however, have been noted within such varieties. In most instances these were not pronounced. Such individuals survived where the majority were killed by the rust. Although they were considerably rusted, the uredia were fewer and smaller than those of the more susceptible individuals. Self pollination and selection for resistance through several generations resulted in the segregation of highly resistant types in which little or no rust developed under the conditions most favorable for the rust.

No attempt was made to keep complete genetical data, since it was possible to devote only a limited amount of time to the problem. The development of sterility as inbreeding progressed also introduced complications and seriously interfered with the selection of lines homozygous for marked resistance. It seems evident that the original selections from the cultivated varieties carried factors for several types of resistance. One of these governing the development of the marked resistance has been isolated by Emsweller and Jones. They have shown that it is inherited as a simple dominant factor, and have successfully utilized it in developing highly rust-resistant commercial types.

It also seems evident that the pronounced resistance occurring in some of the wild species of Antirrhinum and possibly certain wild strains of *A. majus* can be successfully utilized in the development of rust-resistant snapdragons. From the cross, *A. glutinosum* × Giant White Selection, a highly rust-resistant type, approaching Giant White in plant and flower characteristics, has been obtained. The pronounced resistance of this strain is also inherited as a simple dominant factor. White (16), from this same cross, has been successful in developing several commercial types having pronounced resistance. The inheritance of the marked resistance of *Antirrhinum Ibanjezii* B5 as a simple dominant factor when crossed with susceptible commercial strains of snapdragon indicates that the resistance of this species can be similarly utilized.

While the results that have been obtained indicate that resistant strains of snapdragon will probably solve the rust problem, the possibility of physiologic forms in the rust and the complications that might result should not be overlooked. So far, there has been no certain evidence of the occurrence

of such forms. The development of homozygous highly resistant lines of snapdragon now makes it possible to approach this problem with some degree of certainty. In this connection the aecial host also may play an important part. Recent investigations in the rusts all indicate that their sexual reproduction is connected with the aecial stage. The aecia, therefore, may play an important rôle in the development of physiologic forms. Elsewhere (12), it has been shown that *Puccinia Antirrhini* is heteroecious. So far, the aecial stage has not been discovered. In a rust of the type of *P. Antirrhini* it is difficult to foretell the alternate host. Since the rust is apparently native to California, the most promising method would be to locate heavily rusted California species of Antirrhinum and watch the species of other genera in the immediate vicinity for aecia, checking such observations with cultures.

SUMMARY

In these investigations only species of Antirrhinum have been infected with *Puccinia Antirrhini*. Species of Linaria and *Adenostegia pilosa* have been resistant.

The results obtained with species of Antirrhinum are summarized in table 2.

Cultivated varieties of *Antirrhinum majus* have, for the most part, been very susceptible. A few selections were obtained showing some resistance. From these by self pollination and selection, highly resistant types have been obtained.

Highly resistant types also have been obtained from crosses of snapdragon with resistant selections of *Antirrhinum glutinosum* and *A. Ibanjezii*. In each case, resistance has been obtained that is inherited as a simple dominant factor.

The resistance derived by selection from varieties of snapdragons and from the *Antirrhinum glutinosum* cross has been utilized in California by Emsweller and Jones (8) and in Massachusetts by White (16) for developing highly resistant commercial types of snapdragon.

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STUDIES ON THE HOST RANGE OF PHYTOMONAS STEWARTI AND P. VASCULARUM[1]

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INTRODUCTION

The host range of *Phytomonas stewarti* (E. F. Smith) Bergey *et al.*, the causal organism of Stewart's wilt disease of corn,[3] *Zea mays* L., was the subject of studies the results of which are reported in this paper. If hosts other than corn existed, they might be important in the life history of the disease. In order to determine whether some other species might be infected by the Stewart's wilt pathogen, a series of inoculations was made in the greenhouse and in the field upon the following plants: sorghum, *Holcus sorghum* L.; sugar cane, *Saccharum officinarum* L.; Sudan grass, *Holcus sudanensis* Bailey; yellow foxtail, *Setaria glauca* Beauv.; German foxtail millet, *Setaria italica* Beauv., var. *Stramineofructa* Bailey; and common millet, *Panicum miliaceum* L.

The number of bacterial pathogens that cause symptoms on corn similar to those caused by *Phytomonas stewarti* on corn also was investigated.

The similarity between the symptoms of Stewart's wilt and those of Cobb's disease of sugar cane,[6] prompted the writer to test the effect of *Phytomonas vascularum* (Cobb) Bergey *et al.* on corn and subsequently on sorghum.

MATERIALS AND METHODS

Unless otherwise stated, the culture of *Phytomonas stewarti* used in the inoculation tests was a progeny of a single cell. It was the standard culture employed by the writer throughout his previous studies on Stewart's wilt.[7]

[1] Published with the approval of the Director of the Wisconsin Agricultural Experiment Station.

[2] The writer wishes to acknowledge his gratitude to Eugene Herrling for the photographs.

[3] The term "Stewart's wilt" disease in this paper refers to the disease caused by *P. stewarti* as described by Stewart[4] and Smith.[5] The term "bacterial wilt" is used to include Stewart's wilt disease and any other disease expressed by similar symptoms and caused by a bacterial organism or organisms other than *P. stewarti*.

[4] Stewart, F. C. A bacterial disease of sweet corn. New York Agr. Expt. Sta. Bul. 130. 1897.

[5] Smith, E. F. Bacteria in relation to plant diseases. Vol. 3. Washington, D. C. (Carnegie Inst. Wash. Publ. 27). 1914.

[6] Smith, E. F. See footnote 5.

[7] Ivanoff, S. S. Stewart's wilt disease of corn, with emphasis on the life history of *Phytomonas stewarti* in relation to pathogenesis. Jour. Agr. Res. 47: 749–770. 1933.

The cultures of *P. vascularum* were obtained from A. F. Bell of the Bureau of Sugar Experiment Stations, Australia. Their identity was confirmed by cultural and pathogenicity tests. All inoculations were made by means of needle punctures at the base of the plant by employing either a common needle or a plant inoculator.[8] In every inoculation test besides the inoculated plants there were about an equal number of controls. Some were punctured, while othere were left untreated. In all cases both kinds of control plants remained normal and, in the few reisolation tests made, yielded no pathogenic bacterial organisms.

Sugar-cane cuttings of 2 varieties, Co 281 and CP 807, were secured from E. C. Tims of the Louisiana State University. Sorghum seed of the variety Roxorange (Waconia Orange) was secured from A. H. Wright, of the Wisconsin Agricultural Experiment Station. Seed of other sorghum varieties including Blackhull kafir, Darso, Dwarf Yellow milo, Kansas Orange sorgo, Gooseneck, and Red Amber sorgo were obtained from A. L. Clapp, of the Kansas State College of Agriculture and Applied Science. Sudan grass seed of an unknown variety was obtained from a commercial firm. The seed of the Yellow foxtail, common millet, and German millet was obtained from A. L. Stone, of the College of Agriculture, University of Wisconsin. Two varieties of corn were used, Golden Bantam and Golden Gem, both known to be very susceptible to Stewart's wilt.

Isolations of *Phytomonas stewarti* from the tissue of the inoculated plants were made in the usual manner, employing nutrient dextrose agar. The pathogenicity of all isolated cultures was tested by inoculations on corn. For culturing and isolating *P. vascularum,* Wilbrink's medium was ordinarily used, which had the following composition: sucrose, 20 gr., peptone 5 gr., K_2HPO_4 0.5 gr., $MgSO_4$ 0.25 gr., agar 17 gr., and water to make one liter. The medium was adjusted to pH 7.0.

The inoculation tests were started in the greenhouse in the fall of 1933 at Madison, Wisconsin, continued in the field during the summer of 1934, and in the greenhouse at Madison, during the fall of the same year.

INOCULATION TESTS WITH PHYTOMONAS STEWARTI

Corn. The leaf symptoms induced on corn have been described in detail elsewhere.[9] For the purpose of comparison some of these symptoms are illustrated in figure 1, A and B.

Sorghum. In the greenhouse the first symptom appeared about 5 days after inoculation as a reddening of the infected veins (Fig. 1, C). This was followed by the appearance of red semicircular or irregularly shaped areas on either side of the affected veins (Fig. 1, D and E). These areas in

[8] Ivanoff, S. S. A plant inoculator. Phytopath. 24: 74–76. 1934.

[9] Ivanoff, S. S. See footnote 7.

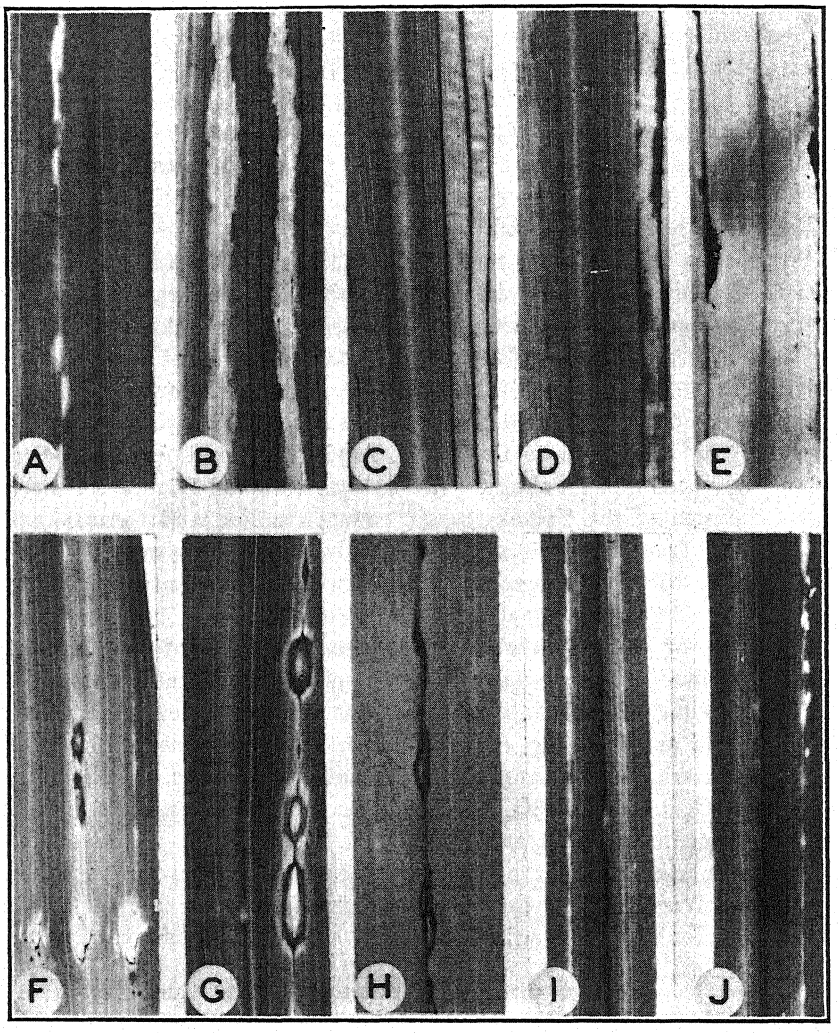


FIG. 1. Leaf symptoms on various plants induced by *Phytomonas stewarti* (magnifications approximate). A. Early stage on corn. ×1. B. Later stage of A. ×1. C. Early stage on sorghum. Red bundles appear black. ×1.5. D. Late stage of C showing invasion of the parenchyma. ×1.5. E. Later stage of D showing invaded parenchyma, red, sharply defined. ×3.5. F. Early stage on Sudan grass (holes at lower end from inoculation). ×1.5. G. Later stage of F. ×1.5. H. Stripe on German millet. ×3. I and J. Stripes on yellow foxtail. ×3.

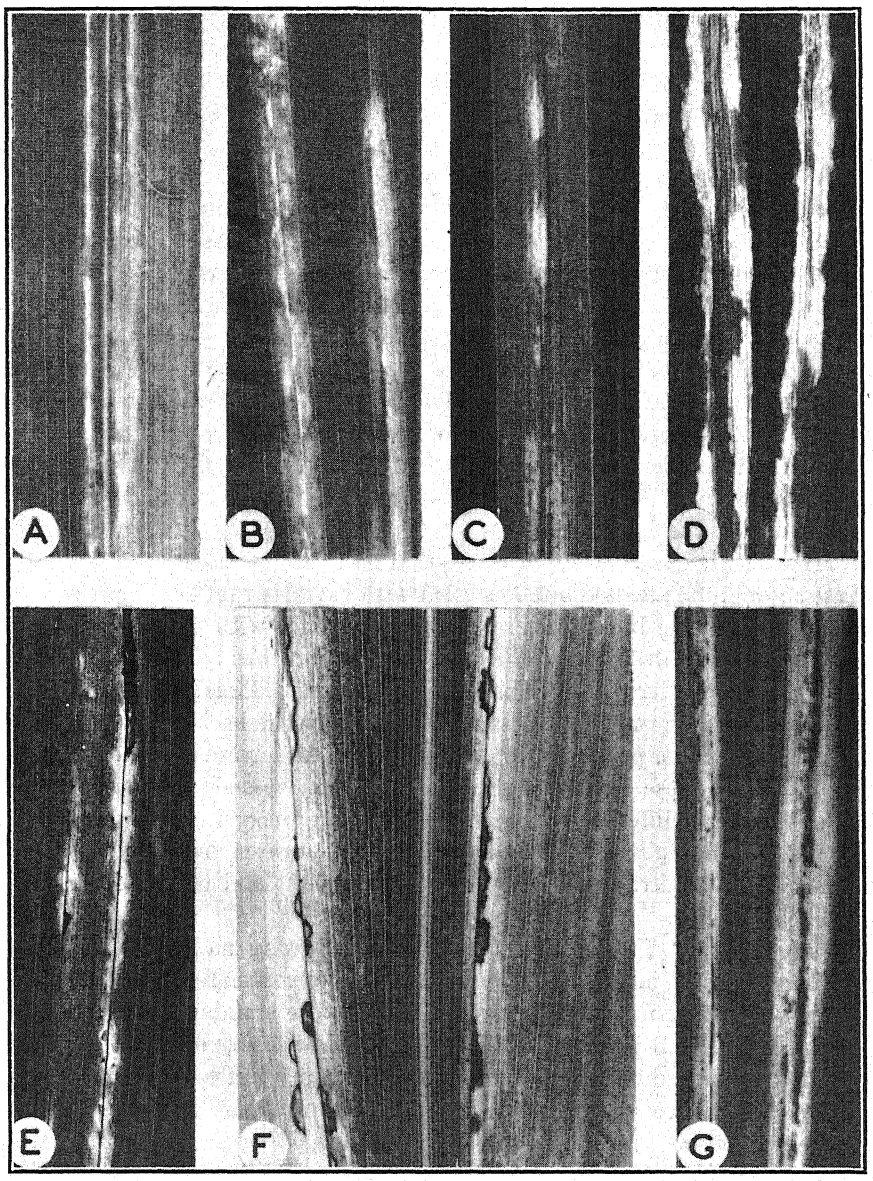


FIG. 2. Leaf symptoms on various plants induced by *Phytomonas vascularum* (magnifications approximate). A. Early stage on corn. ×1. B. Later stage of A, showing semitransparent water-soaked areas in the parenchyma. ×1. C. An enlarged view of semitransparent water-soaked areas along the veins of corn. ×3.5. D. Later stage of B. ×1. E. Early stage on sorghum. ×1.5. F. Later stage of E, showing characteristic red, semicircular areas along the veins. ×2.5. G. Stripes on sugar cane. ×1.

most cases were sharply delimited (Fig. 1, E), but occasionally the red pigment seemed to have diffused into the green tissue without leaving any definite demarcation (Fig. 1, D). The tissue adjoining the sharply delimited red areas usually lost its normal green color and became pale green to yellow. In some cases the inoculations caused wilting of entire leaves. Such symptoms were induced on plants belonging to all the varieties mentioned earlier and by more than one strain of the Stewart's wilt organism.

In the field only 2 varieties of sorghum were inoculated: Roxorange and Kansas Orange sorgo, the former being earlier and shorter in height than the latter. Over 1000 plants were inoculated twice, the first time about 5 weeks after planting and the second time about 7 weeks. Both the standard culture of *Phytomonas stewarti* and a mixture of strains, respectively, were employed. No leaf symptoms developed as a result of these inoculations. The stalks of Roxorange sorghum, however, developed red bundles that were plugged with bacteria and, in a few cases, formation of small cavities in the pith was observed. Kansas Orange sorgo, with a few exceptions, remained unaffected by the inoculations.

Microscopic examination of leaves that had become diseased in the greenhouse showed the affected bundles filled with bacteria that oozed out in large masses when these bundles were placed in contact with water. On either side of these bundles bacterial pockets were found that corresponded to the semicircular and irregularly shaped areas mentioned earlier. These pockets, like those in corn,[10] doubtless appeared where the bacteria escaped from the vessels into the parenchymatous tissue. In the manner of development and in shape, these abnormal areas on sorghum resembled these on corn. The outstanding difference between the leaf symptoms on sorghum and those on corn was the light red color of the infected sorghum tissue. The stripes on corn were semitransparent by transmitted light and tan to light brown by reflected light.

Sudan Grass. Two series of field inoculations were made on Sudan grass. The first series of inoculations were made on approximately 300 5-week-old plants with a mixture of 5 cultures, including the standard culture. As a result, only a small percentage of the plants showed any symptoms. These consisted in the reddening of the invaded vascular bundles of the leaves and stem and of a discoloration of the parenchymatous tissue along the infected leaf veins, similar to that in the case of corn. Although the bacteria were reisolated from several plants these symptoms were not considered striking. The second series of inoculations were made later in the season on about 100 3 to 4-week-old Sudan grass plants of a later planting with the standard culture. Disease symptoms developed on each of the inoculated plants but not on the controls. The symptoms that developed upon the leaves (Fig. 1, F and G) resembled those induced on sorghum by the red color of the

[10] Ivanoff, S. S. See footnote 7.

stripes and by the discoloration of the parenchymatous tissue along the affected veins. In many cases 2 of the semicircular areas occurring on both sides of a vein had a common center that resulted in the formation of elliptical spots along the vein (Fig. 1, G). The central portion of these spots in time became necrotic and turned light brown in color. In some cases the dry necrotic tissue fell out and left an elliptical hole in its place (Fig. 1, G). About 3 weeks after the inoculation, some of the stems were cut open and were found to contain some red bundles from which the bacteria were reisolated.

Common and German Millets. Most of these inoculations were made in the greenhouse with the standard culture of *Phytomonas stewarti* and with a mixture of strains of the same organism. The stripes that developed upon the leaves resembled in color, shape, and manner of development, more closely those produced on corn than those on any other plant tested. The only important difference was in the relatively narrow width of the stripes on millet, which probably was because of the narrower host leaves (Fig. 1, H).

Yellow Foxtail. The leaf stripes induced on this host were characterized by being very narrow, well defined, and a dull white or tan color (Fig. 1, I and J). Although no well defined semicircular, water-soaked areas along the veins have been observed to precede the formation of these stripes, the evanescent quality of such areas may have been missed because of the smallness of the leaves.

Sugar Cane. About 25 sugar-cane plants were inoculated in the greenhouse with 2 strains of the Stewart's wilt organism when the plants were 4 to 5 weeks old. Although the results were negative, the extent of the trials does not justify the conclusion that sugar cane is immune.

Cross inoculations were made by using the organism reisolated from sorghum upon corn, Sudan grass, and the millets, and *vice versa.* No change in the pathogenicity of the bacteria was observed as a result of the passage through other plants.

The existence of hosts to *Phytomonas stewarti,* other than those already named, is thought to be very probable.

The natural occurrence of the disease upon these hosts in the field has not yet been observed by the writer. An important reason may be the fact that during the summer of 1934 the incidence of Stewart's wilt on corn in northern Illinois and Wisconsin, where these observations were made, was negligible. On the most susceptible open-pollinated varieties of corn the disease ranged from 0 to a fraction of 1 per cent.

INOCULATION TESTS WITH PHYTOMONAS VASCULARUM

Greenhouse inoculations on corn and sorghum with *P. vascularum* revealed 2 things: firstly, that this microorganism causes disease upon these

plants, and secondly, that the symptoms induced by it are so similar in appearance to those induced upon the same hosts by *P. stewarti* that, so far, they have been differentiated with difficulty, if at all.

Corn. Three types of symptoms were induced by inoculating corn with *Phytomonas vascularum:* water-soaked stripes along infected leaf veins, cavities in the stalk, and general stunting.

The leaf stripes (Fig. 2, A–D) developed just as did those caused by *Phytomonas stewarti,* with this exception, that the incubation period in the greenhouse in the case of *P. vascularum* was about twice as long as that of Stewart's wilt. In addition, the water-soaked areas induced by the sugarcane organism assumed in time a somewhat rusty color, while those of Stewart's wilt were light brown. A third difference was the appearance of numerous, small, circular, elliptical, or irregular, water-soaked areas in the parenchymatous tissue of the leaf, away from the vein infected by *P. vascularum* (Fig. 2, B), and their absence in the case of *P. stewarti.* In each case these differences in the leaf symptoms caused by the 2 species of bacteria could be observed only at the right time during the course of their development and upon close examination. A glance at the respective symptoms at a late stage of development would commonly fail to differentiate them.

Cavities in the stalk of Golden Gem sweet corn were observed in some cases about 5 weeks after inoculation with *Phytomonas vascularum.* The development of these cavities started with the appearance of water-soaked tissue at the terminal bud of the culm and at the ear bud on the side of the culm (Fig. 3, G). Later the water-soaked tissue became very soft, changed to light brown, then to dark brown. Then the cavity appeared, apparently as a result of the digestion of the tissue by the bacteria (Fig. 3, H). The size of these cavities enlarged in length and width with the elongation of the stem internodes. In development and appearance these cavities resembled those induced on the variety Leaming of field corn inoculated in the field with a strain of *P. stewarti* (Fig. 3, A–D). In addition to the organism introduced, some other bacteria were isolated later from these cavities in great abundance. The effect of these secondary organisms on the size and consistency of the rotted tissue in the stalk remains to be investigated both in the case of *P. vascularum* and of *P. stewarti.*

Dwarfing and stunting of Golden Gem corn plants inoculated with *Phytomonas vascularum* probably is due to the activities of the bacteria in all of the infected tissue and particularly because of the cavities previously mentioned. Inoculated plants that developed leaf symptoms but did not develop cavities appeared only slightly smaller than the punctured controls. On the other hand, plants that developed cavities were considerably smaller than the control plants and their nodes were much closer together than were those of the controls.

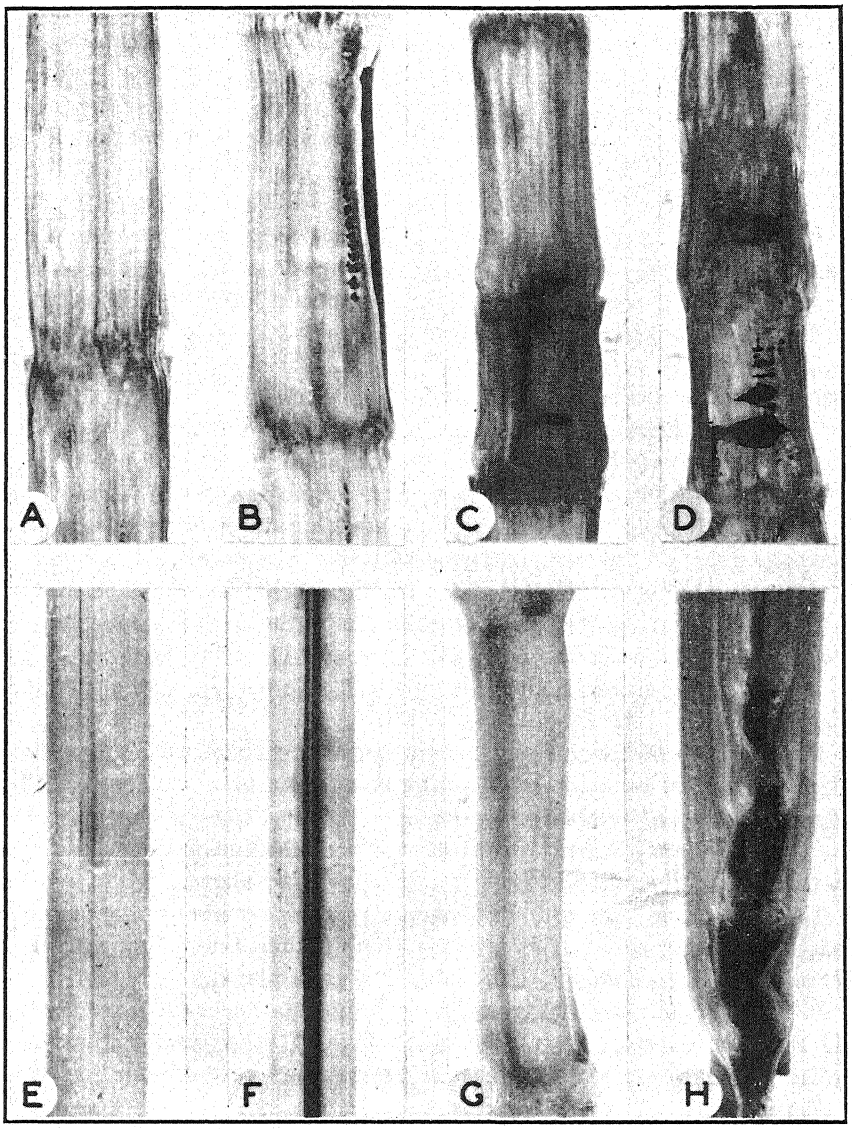


FIG. 3. Stalk rot of corn and sorghum induced by *Phytomonas stewarti* and *P. vascularum*. A–D. Four stages in the development of stalk rot of Leaming field corn, induced by a strain of *P. stewarti*. Color of cavity wall light to dark brown. ×1. E–F. Two stages in the development of stalk rot of sorghum induced by *P. vascularum*. Color of infected tissue deep red. ×2 and ×1.5, respectively. G–H. Two stages in the development of stalk rots of Golden Gem sweet corn, induced by *P. vascularum*. Color of infected tissue light to dark brown. ×1.5.

Sorghum. Roxorange sorghum plants, when inoculated in the greenhouse with *Phytomonas vascularum,* developed leaf symptoms closely resembling those induced on the same host and on corn by *P. stewarti.* In addition the sugar-cane organism induced rotting and cavities in the sorghum stalk of a type that seemed to be characteristic for that host.

The leaf stripes, in general, were similar in shape but not in color to those induced on corn by *Phytomonas stewarti* (Fig. 2, E and F). The color of the infected veins and the semicircular areas in the parenchymatous tissue along the vein was red, making the stripes conspicuous. In addition to the red tissue there was the usual discoloration of the green tissue bordering the red areas where the bacterial pockets were located.

Reddening of vascular bundles in the stalk, rotting of the pith and formation of cavities in the stalk resulted in some cases from the inoculation with the sugar-cane organism (Fig. 3, E, F). These cavities had 2 outstanding characteristics in color and shape that distinguished them from the cavities produced in corn by *Phytomonas vascularum* and *P. stewarti* and from the small pockets in the sorghum stalk produced by *P. vascularum.* First, the color of the decayed tissue of these cavities was deep red, more intense than the light red color of the small pockets formed in the sorghum stalk by *P. stewarti,* and second, these cavities in the early stages of development were straight, regular, long and narrow. They extended throughout the length of the several nodes, with parallel side borders strikingly delimiting the affected pith tissue (Fig. 3, F).

For the sake of comparison, inoculations were made in the greenhouse with *Phytomonas vascularum* on young sugar-cane plants. The usual leaf stripes appeared after about 2 weeks (Fig. 2, G). These stripes have been described previously[11] and are illustrated here for convenience in making comparisons with similar stripe symptoms described above.

The inoculation tests with *Phytomonas vascularum* were limited to sugar cane, corn, and sorghum. It is likely that further study may extend the host range of *P. vascularum* to include other grass plants.

In view of the results obtained in these inoculation tests, it seems probable that an investigation of the host range of other bacterial parasites known to induce stripe symptoms on grasses may reveal other important relationships among these parasites.

DISCUSSION

For the sake of clarity the foregoing results have been rearranged as follows:

The symptoms induced upon the leaves by either of the 2 pathogens on any of the hosts mentioned had some common resemblances and some individual distinguishing characteristics, depending upon the host.

[11] Smith, E. F. See footnote 5.

The similarity features of these symptoms were the formation of stripes along the veins in a characteristic manner, already described in the case of Stewart's wilt of corn,[12] namely, by the change of color of the affected vein for some distance above and below the point of entrance of the bacteria; by the appearance of semicircular or irregularly shaped areas on either side of the vein, with a consistency and color depending upon the host; and by a general discoloration of the green parenchymatous tissue adjoining these areas.

The differences thus far noted as to the appearance of the leaf symptoms in the various hosts consisted in the color of the veins and of the semicircular or irregular areas adjoining the affected veins, in the consistency of the tissue of these areas, in the arrangement of these areas, and in the duration of the incubation period.

The color of the affected veins and that of the semicircular or irregular areas of plants inoculated with *Phytomonas stewarti* was tan to light brown in the case of corn, yellow foxtail, and the millets, and red in the case of sorghum, and Sudan grass. The color of the same infected tissue of plants inoculated with *P. vascularum* was light brown to rusty in the case of corn and red in the case of sorghum. The consistency of all light brown areas, just mentioned, in the case of all hosts and both pathogens, was at first water-soaked. These areas later became necrotic and dry. The red areas in all hosts did not appear water-soaked probably because of the obscuring effect of the red pigment.

The arrangement of the semicircular or irregular areas adjoining the affected veins was, in all cases but one, in a more or less alternating manner. In the case of Sudan grass inoculated with *Phytomonas stewarti,* very often two of these areas occurred opposite each other, thus forming a more or less round or elongated spot that had its center at the vein.

The incubation period in the case of corn inoculated in the greenhouse with *Phytomonas vascularum* was twice as long as in the case of the same host inoculated with *P. stewarti.*

Rotting of the corn and sorghum stalk tissue in various degrees was produced by *Phytomonas stewarti* and *P. vascularum.* The affected sorghum tissue developed a red color of different intensities, according to the pathogen introduced. In the case of *P. stewarti* the color of the diseased tissue was light red; in that of *P. vascularum* it was deep red. No striking differences thus far have been observed in the stalk rots produced on corn by the 2 pathogens.

The most favorable stage of host development for infection and the comparative susceptibility of the various hosts to both pathogens have not been determined.

[12] Ivanoff, S. S. See footnote 7.

SUMMARY

Symptoms are described that were induced by field and greenhouse inoculations with strains of *Phytomonas stewarti* on corn, *Zea mays;* sorghum, *Holcus sorghum;* Sudan grass, *Holcus sudanensis;* yellow foxtail, *Setaria glauca;* German foxtail millet, *Setaria italica* var. *stramineofructa;* and common millet, *Panicum miliaceum,* and induced by greenhouse inoculations with *P. vascularum* on corn, sorghum, and sugar cane, *Saccharum officinarum.*

The leaf symptoms induced by the Stewart's wilt organism on sorghum, Sudan grass, yellow foxtail, German and common millets, with a few exceptions noted, resembled in shape and manner the development of those induced by the same pathogen on corn.

The sugar-cane organism induced symptoms upon the leaves of corn and sorghum that resembled those induced on the same hosts by Stewart's wilt pathogen.

The sugar-cane organism, in addition, induced a rotting of the corn stalk similar to that induced by *Phytomonas stewarti* on the same host. *P. vascularum* also induced cavities in the sorghum stalk.

NEW ECONOMIC HOSTS OF THE STEM- AND BULB-INFESTING NEMATODE

G. H. GODFREY AND C. EMLEN SCOTT[1]

(Accepted for publication January 21, 1935)

Steiner and Buhrer,[2] Cobb and Steiner,[3] and Goodey(5) have listed the hosts of the stem- and bulb-infesting nematode, *Anguillulina dipsaci* (Kühn) Gervais and van Beneden, and have shown a known host range of some 240 species of plants, about 30 of which are cultivated food plants. The present paper gives an account of the occurrence of this nematode on 3 hitherto (4) unreported food-plant hosts: salsify, *Tragopogon porrifolius* L.; parsley, *Petroselinum hortense* Hoffm.; and celery, *Apium graveolens* L. The first of these belongs to the family Compositae, the others to the Umbelliferae.

HISTORY OF OCCURRENCE AND SYMPTOMS OF THE DISEASE

In April, 1933, specimens of diseased salsify plants were received by the Division of Plant Pathology, University of California at Berkeley, from the vicinity of San Juan, San Benito County. Symptoms suggested stem- and bulb-nematode infestation, and microscopic examination verified this diagnosis. The nematodes were found in great abundance in swollen leaf bases, in young leaves just issuing from the crown, and here and there in swellings in leaf blades. The infected tissues in the crown region were cream color, becoming brown, instead of the normal white and light green. In tissues showing advanced stages of injury, some necrosis occurred, followed by secondary organisms. Nematodes were present in all stages, eggs, larvae of different ages, and adults. Measurements of adult males and females were typical for the species as described by Cobb (1) and in great detail by Goodey (5).

In late April the writers visited the field whence the specimens had come, one of many there devoted to seed production. The well-established salsify plants had been transplanted from a seed bed in the fall of 1932, and their flower stems were just beginning to shoot. The field had been devoted to

[1] Grateful acknowledgment is made to Professor W. B. Herms, Division of Entomology and Parasitology, University of California, for materials and equipment used in connection with the studies herein reported, and for making possible the photographs used for illustrations.

[2] Steiner, G., and E. M. Buhrer. A list of plants attacked by *Tylenchus dipsaci*, the stem or bulb nema. U. S. D. A., Bur. Plant Indus., Plant Dis. Rptr. **16**: 76–85. 1932.

[3] Cobb, Grace Sherman, G. Steiner and F. S. Blanton. Observations on the significance of weeds as carriers of the bulb or stem nematode in narcissus plantings. U. S. D. A., Bur. Plant Indus., Plant Dis. Rptr. **18**: 127–129. 1934.

garlic the year before, and, significantly, the bulb nematode was reported present in that crop. About 10 per cent of the salsify plant spaces were vacant, the plants having been rogued out and destroyed because of disease. An additional 5 per cent (estimated) had unmistakable symptoms of the nematode disease. Since other diseases, including a heart rot caused by *Sclerotinia libertiana* and a yellow dwarfing disease suggestive of aster yellows were present, it is probable that only a part of the failures noted were due to the nematode disease.

Adjacent to the salsify field were fields of garlic showing also incidence of nematode injury, ranging from 2 to 10 per cent in some areas. The

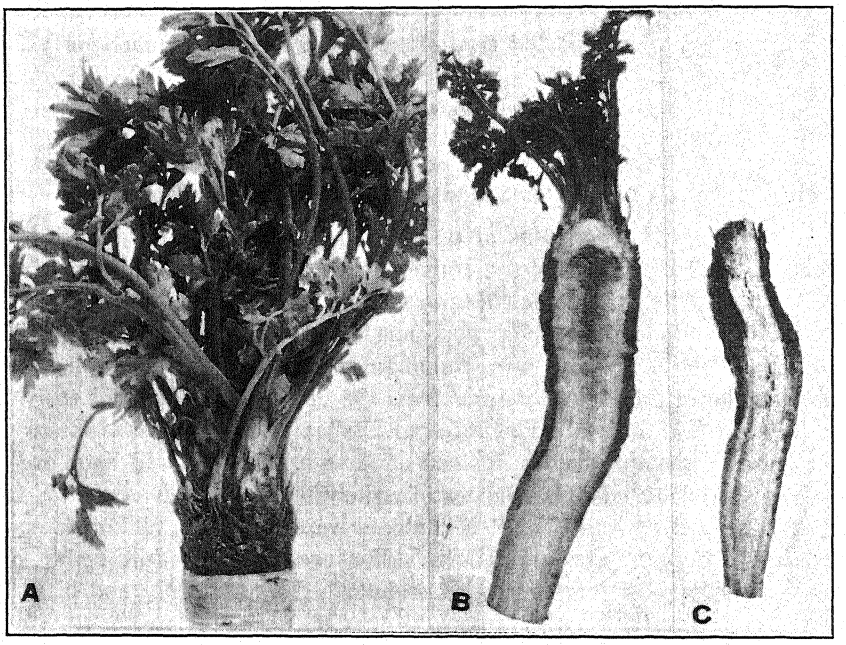


FIG. 1. A. A stem-nematode-infested Hamburg parsley plant, showing necrosis and shredding of leaf-petiole bases and yellowing of outer leaves. Leaf petioles showing such symptoms are interiorly discolored and nematode invasion extends even into the leaflets. $\times \frac{3}{4}$. B. and C. Hamburg parsley roots showing typical symptoms of stem-nematode attack. B. Invasion probably directly from the soil and extending into the central part of the root. C. Invasion probably in part, at least, from above, for complete necrosis had occurred in the crown region. In both the nematodes extend considerably beyond the visibly infested portions.

variety known as Late garlic was much more highly susceptible than Early garlic, though diseased plants of the latter, definitely in evidence, were marked by a progressive yellowing or browning of lower leaves. Close examination disclosed unmistakable symptoms of nematode invasion, *i.e.*,

thickened scales at the leaf bases, sometimes with splitting, and eventual necrosis in the more severe cases. It was to be noted that no "spikkels" or swollen areas of yellowish coloration occurred on the leaf blades, such as is seen in Narcissus. Subsequent laboratory examination showed the nematodes to be exceedingly abundant in the yellowed leaves, in the bulb scales, and even in green leaves showing no obvious symptom. The occurrence of this nematode in garlic and onions is well known.

A near-by parsley field also showed serious nematode injury, which was even more pronounced than in the salsify. An average of 10 per cent (rough estimate) of the plants were clearly affected. The symptoms resemble those caused by the same nematode in alfalfa and clover, *i.e.*, dwarfing of leaves and leaflets, "tulip-root" effect in leaf petiole bases, distorted regions in the leaves; though, in general, this macroscopic symptom so common with other hosts was conspicuously rare. In severer cases leaf petiole bases were split and a brownish necrosis in the interior region was evident.

The most striking case of the disease ever noted by the writers in any crop was found in a field of Hamburg parsley, some 5 or more acres in extent, about a mile distant from the first field. This, too, had been planted for seed production. At first glance it was evident that more than 50 per cent of the plants were missing. Many of those remaining were dwarfed and off-color, and still others showed the leaves wilted to the ground. Mildly affected plants showed symptoms similar to those described above for plain parsley. Figure 1, A illustrates a typical specimen, with petiole bases swollen and split, brown within, and outer leaves turning yellow. More extreme cases showed severe necrosis at the point of attachment of the leaves. When attempts were made to pull such plants for examination, the leaves would break off, leaving the roots in the ground.

Root Symptoms in Parsley. Such roots, when removed, showed necrotic regions extending well into the roots. In some cases such regions distinctly extended downward from the crown, and frequently the central region as well as the cortical region of the roots was so affected. In other cases, as shown by sectioned roots, cortical necrosis occurred apparently independently of the crown. It was not until such roots were examined in the laboratory that it was definitely determined that the discoloration in roots was accompanied by extremely heavy invasion by nematodes. These organisms appear to have found a highly favorable medium for growth and reproduction in the parsley roots, invading even white portions of the root beyond the evident discolored region. The evidence indicated that invasion of roots frequently took place directly from the soil and was quite independent of leaf invasion. Figure 1, B and C, shows 2 parsley roots, the first with independent root invasion and the other with the greater part of the injury apparently progressing downward from the crown.

Approximately a month after the first field observation, a second visit was made to these fields. It was then evident that the nematode disease was progressing rapidly in all diseased plants, but particularly so in the Hamburg parsley. An exact count was made in 4 representative 600-foot rows of this crop and it was determined that almost exactly 80 per cent of the stand was either missing (having been rogued out and destroyed) or dead. Of the remaining plants many were dwarfed and yellowed, and it was obvious that they, too, would perish before seed production. The field, therefore, was virtually a total failure.

OBSERVATIONS ON THE SUSCEPTIBILITY OF OTHER PLANTS

Adjacent to the Hamburg parsley were fields of salsify, Early and Late garlic, and double- and triple-curled parsley, all with occasional plants showing nematode infection, but much less than did the Hamburg parsley. Either this plant was distinctly much more susceptible than were the other plants, or the field in which it was planted was initially more heavily infested than were the surrounding areas. One other hitherto reported host, sweet pea, *Lathyrus odoratus* L., planted for seed production in a near-by field, likewise showed an occasional plant with typical nematode disease symptoms and definite nematode invasion. Orchard morning-glory, *Convolvulus arvensis* L., which grew as a weed in the most heavily infested areas, was apparently entirely free, although it has been reported as a host.

The occurrence of the disease in the third new host, celery, was not observed in the field, but rather in cultures of the plant in pots, following artificial greenhouse inoculations with colonies of nematodes from garlic and parsley, as described elsewhere in this paper.

THE CAUSAL ORGANISM

By way of substantiation of the identity of the nematode, measurements according to Cobb's formula are given of specimens taken from Hamburg parsley, as follows:—

Females, average of 14 adults,

.8	7.5	♀	81.5	93	
.8	1.9	2.3	2.4	1.3	1177 μ

Males, average of 8 adults,

.8	7.5	♀	M	93.8	
.8	1.8	2.1	2.2	1.5	1131 μ. Spicule, 22.1 μ

Even though the proportional distance to the intestine is omitted in both cases because of the obscurity at this point in the material examined, other measurements and anatomical details check so closely with accepted descriptions that there can be no doubt as to the identity. Random measurements from the other newly reported hosts checked equally well, and are not reported.

LOCATION OF NEMATODES IN DISEASED TISSUES

In the course of laboratory examinations of diseased material it was found that nematodes in great abundance permeated tissues in which outward manifestation of the disease was evident. In addition, border tissues not showing injury, and sometimes tissues several inches away from obvious injury, were found to be swarming with nematodes in all stages of development. In garlic, unswollen leaves of normal color above infested scales contained hundreds of them (3). Dissection of parsley leaves showing swellings in petiole bases and internal discoloration disclosed the upward extension of the internal petiole browning, sometimes into leaflet petioles, and that such regions were invaded throughout by nematodes. More than this, the leaflets themselves were frequently invaded, even in the mesophyll. Figure 2, A shows a typical leaflet cleared in clove oil with the nematodes stained to show them in natural position, following a special procedure described elsewhere (3). Such leaflets showed no distortion. When heavily infested they were slightly yellowed, sometimes mottled, and, like the rest of the leaf and the entire plant, were somewhat undersized. The path of leaflet invasion was undoubtedly the interior region of the leaf petiole, and midrib, though the latter showed no browning or distortion. Figure 2, B, a 1-millimeter section of infested Hamburg parsley root, similarly prepared, shows the extremely heavy invasion and reproduction that occur in this region of the plant. This heavy root infestation is unusual for the stem- and bulb-infesting nematode. The nematodes apparently migrate very readily through the tissues; they are found in abundance in the advancing margin of visible infestation, and even in the healthy white tissues just beyond. Occasional roots showed infestation within the central region, apparent invasion having occurred through the stele from the cortical tissues.

No plants of any of the hosts herein reported were seen in the process of seed development. It is therefore impossible to say whether or not seed invasion occurs, such as has been reported for *Hypochaeris radicata* and *Taraxacum officinale* and other hosts (2, 6). In garlic, where propagation is largely by means of bulblets (cloves), it is obvious that much spread by this means might readily occur.

EXPERIMENTAL INOCULATIONS

A simple experiment was conducted to determine in part the relationship of the particular strain of *Anguillulina dipsaci* found in these hosts, to other known strains. Only one other certain stem nematode strain, that in alfalfa, was immediately available. It was acquired in typical diseased plant materials from the Agricultural Commissioner of San Benito County. Another nematode, described tentatively by Steiner and Scott (7) as *Anguillulina dipsaci* var. *amsinckiae,* was collected in abundance in the galled seed heads

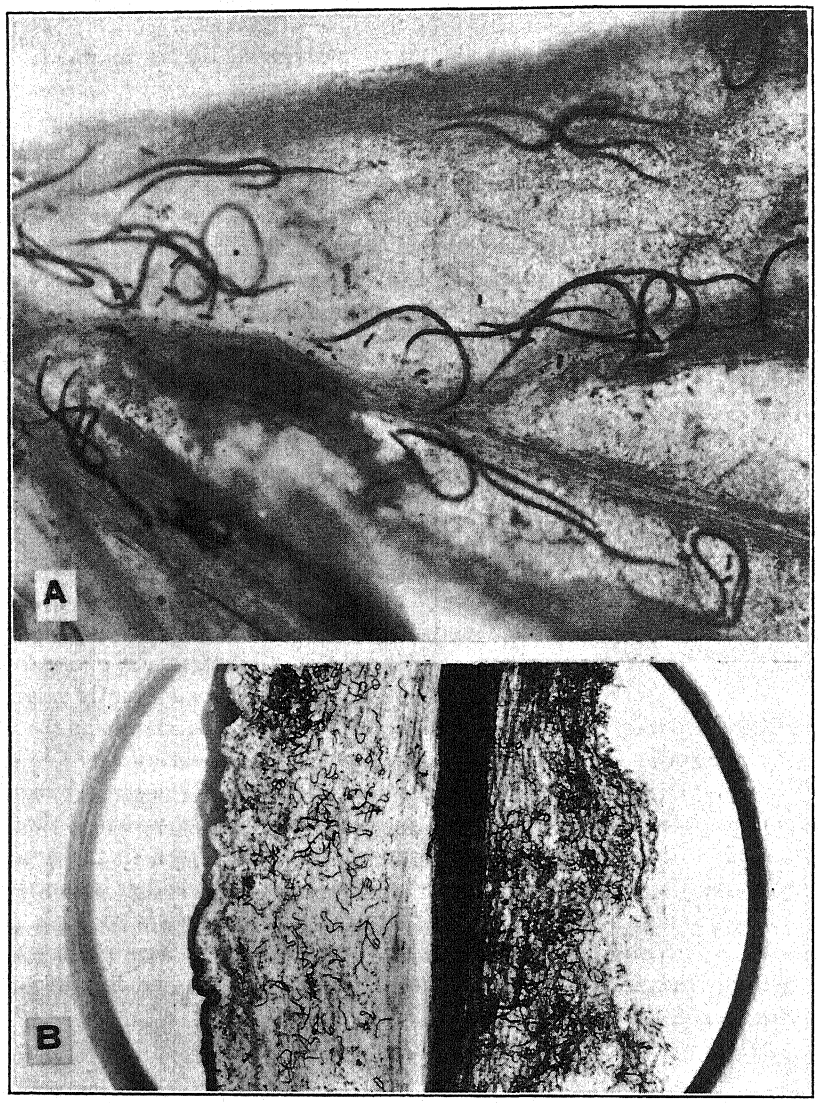


FIG. 2. A. Portion of a leaflet of Hamburg parsley (*Petroselinum hortense*) invaded by stem nematodes (*Anguillulina dipsaci*) without causing distortion. Eggs, larvae, and adults are present mostly in the spongy parenchyma of the leaf mesophyll. ×about 30. B. A section about 1 mm. thick from a root of Hamburg parsley invaded by stem nematodes. Both the cortex and the central region are invaded. ×about 6.

of fiddle-neck, *Amsinckia intermedia* F. and M., from the roadside south of San Jose, and used in the inoculations. These 2 strains, then, with the nematodes from garlic and from parsley, made 4 strains of the organism that were used to inoculate 4 series of large pots of soil. Rather heavy inoculations consisting of broken bits of fresh heavily infested plant materials, were applied in every case by stirring them into the upper 2 inches of soil. In each series were planted seeds of alfalfa, celery, parsley, salsify, and Amsinckia, and bulblets of garlic. The pots were kept moist through several months and frequent observations were made. The Amsinckia seeds were collected from the current season's growth and remained dormant, so no readings were possible on that plant. All other plants grew well. Final conclusions from the experiment are given in table 1.

TABLE 1.—*Results of a cross-inoculation experiment with Anguillulina dipsaci obtained from different sources*

Nematode sources	Presence or absence of infection in different plants tested				
	Alfalfa	Celery	Garlic	Parsley	Salsify
Alfalfa	+++	–	–	–	–
Garlic	–	++	+++	+++	++
Parsley	–	++	+++	+++	++
Amsinckia	–	–	–	–	–

Bur clover, *Medicago denticulata* Willd., listed by Goodey (6) as a host, which appeared as a weed in most of the pots, was likewise negative throughout the period of testing. It would appear that the garlic, parsley, and salsify strains were identical, all probably the original garlic strain of previous years; and that this was distinctly different from the alfalfa strain. It appears also that the Amsinckia strain or variety was distinct from either of the others. In this case an extremely high abundance of infective material was used throughout as inoculum, since every seed-head gall was literally swarming with larvae and dozens of galls were used in every pot. The celery plants, which showed positive infection by the garlic and parsley strains, showed symptoms similar to those in parsley, with swollen petiole bases, some necrosis, and invasion even of leaflets. Celery, parsley, and salsify, then, must be considered as inherently susceptible vegetables in any cropping scheme to follow nematode-infested crops of garlic.

NOTE ON THE LONGEVITY OF THE GARLIC NEMATODE IN SOILS

No experiments in this connection were conducted, but one observation seems worthy of record. The leading garlic grower in the vicinity of San

Juan showed us a garlic field in which the crop had been discontinued for a period because of the high incidence of the nematode disease. The field was devoted for a period of 6 successive years to a non-susceptible crop, lettuce. In the fall of 1932 garlic was again planted, and there was a healthy stand throughout. After careful searching, not a single nematode-infested plant was found. The 6 years in immune crops were apparently sufficient to eradicate the nematode from the field. It is very possible, of course, that a carefully conducted experiment with different rotation periods would show that a shorter period would suffice to do the same thing.

SUMMARY

Salsify, *Tragopogon porrifolius,* parsley, *Petroselinum hortense,* and celery, *Apium graveolens,* are reported as heretofore unrecorded food-plant hosts of the stem- and bulb-infesting nematode *Anguillulina dipsaci.* Symptoms in all alike were swollen leaf bases, "tulip root" effect in young shoots, yellowing and dwarfing of plants, and some necrosis in the crown region. Hamburg parsley was attacked particularly badly, a 5-acre field being practically a total failure. An additional symptom in this plant was a brown necrosis of the fleshy roots extending into both cortical and central regions. Invasion of leaflets, without distortion, was likewise demonstrated. The disease in salsify and parsley was found in plantings made for seed production in the vicinity of San Juan, San Benito County, California, in areas formerly in garlic, *Allium sativum,* in which the nematode disease was known to have been present. Diseased celery was not found in the field, but rather in cultures of this plant in pots following artificial greenhouse inoculations with colonies of nematodes from garlic and parsley.

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CULTURAL VARIATIONS OF THIELAVIOPSIS BASICOLA[1]

E. M. JOHNSON AND W. D. VALLEAU

(Accepted for publication January 10, 1935)

Eleven cultures of *Thielaviopsis basicola* (Berk.) Ferraris, the causal organism of black root rot of tobacco, were isolated in June, 1930, by Gilbert's (3) method, from rotting roots of White Burley tobacco from 2 different fields on the Experiment Station farm at Lexington, Kentucky, where tobacco had been grown continuously for several years, and from a field in Woodford County, Kentucky. Seven cultures came from one field on the experiment station farm, 2 from the other, and 2 from Woodford County.

After 2 weeks of growth on potato-dextrose agar in tubes, the 11 cultures differed from each other in color and rate of growth. They were transferred when 2 weeks old to potato-dextrose agar in Petri dishes and kept for 2 weeks at 27° C. Each colony was uniform but most of the cultures differed in rate of growth, color, hyphal, and colony characters, and in spore characters. The 2 almost identical Woodford County cultures were dark olive (5)[2]; the aerial mycelium was deep grayish olive and powdery, and formed concentric rings. The experiment station cultures all differed and none resembled the Woodford County ones. The Station cultures were various shades of olive and brown and differed in appearance and rate of growth of the mycelium and in spore characters. Some cultures produced endoconidia almost exclusively, while in others chlamydospores predominated. One culture, which produced only chlamydospores, was subcultured 2 years and never was observed to produce endoconidia. When the 11 original cultures were 3 weeks old they were again transferred to plates of potato-dextrose agar and grown for 2 weeks at 27° C. The differences were more striking than in the first plating. Most cultures had at least one sector, some had 3 or 4, which differed from the rest of the colony. The variants appeared to have originated in the culture tubes, but became evident in the culture dishes.

SINGLE-SPORE CULTURES

The variations observed might have been due to mixtures in the original isolants. To eliminate this possibility, 26 single-endoconidium cultures were made from the original isolants by Ezekiel's (1) method. Two hyphal-tip cultures were made of the chlamydospore strains because of failure of chlamydospores to germinate readily on culture media. In the first plating

[1] The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the Director.

[2] Ridgway color standards used in descriptions.

FIG. 1. Single-spore cultures of *Thielaviopsis basicola* after 11 days' growth on potato-dextrose agar. A and B. Same culture from different parts of the tube. C (224: 2: w), D (224: 2: y) and E (224: 2: z). From station farm. F and G. From Woodford County. H. Single-spore albino culture, which failed to develop color during 4 years' propagation.

on potato-dextrose agar 8 Woodford County single-spore cultures appeared almost identical and were similar to the original isolants from this field. Twenty station single-spore cultures differed from the Woodford County ones and from one another in color, rate of growth, hyphal characters, and in the ratio of the 2 kinds of spores. The station cultures were deep olive, dark olive, olivaceous black, plumbaceous black, cinnamon brown, and olivaceous brown. Some had abundant white and cottony aerial mycelium, while in others it was olivaceous gray and powdery, with variations between these extremes. In this plating very few sectors developed, the cultures having been in tubes for only 12 to 15 days. Seven single-spore cultures are shown in figure 1. In a second plating, made when the tube cultures were 27 to 30 days old, there were few cultures that did not have one or more sectors. The 2 hyphal-tip chlamydospore cultures, neither of which produced endoconidia, were strikingly different. One was dark olive with dense, white, cottony, aerial mycelium and had numerous large, 3- to 7-septate chlamydospores. The other was Dresden brown to Saccardo's umber; had dense, grayish, cottony, mycelium; and the chlamydospores were smaller and less numerous. The original 26 single-spore cultures and the hyphal-tip chlamydospore cultures, all grown in tubes 30 days, and subcultures from sectors in the previous plating, were again plated on potato-dextrose agar. So many culturally distinct sectors appeared that it was decided to confine further studies to 2 single-spore cultures from different sources.

Twenty-three plates of potato-dextrose agar were inoculated with a single-spore, dark olive culture (224:1:b, station farm) that had been in a tube for 60 days. Twenty-eight sectors that differed from one another developed. They were whitish, grayish, and various shades of olive and brown, and the aerial mycelium varied from dense and cottony through sparse and powdery to almost resupinate. Subcultures were made from all sectors that came to the margins of the colonies. These and the original single-spore culture (224:1:b) were again plated and subcultures were made from all sectors that appeared different. The subcultures were all found to be unstable and formed sectors. Sixty-six culturally distinct variants, which were deep, dark, grayish, ochraceous, and blackish olive, mummy, Vandyke, Sayal and Dresden brown, Saccardo's umber, sepia, ochraceous, cinnamon buff, clay, and white, developed in the 3 series of platings of single-spore culture 224:1:b. There were also variations in type of mycelium, rate of growth, colony, and spore characters.

The following example illustrates the apparent lack of a definite trend in color variation: From a single-spore dark olive culture (224:1:b) a whitish sector resulted in whitish and dark olive sectors; one of these whitish sectors produced dark olive and a Dresden brown sector. A grayish sector from the original dark olive culture resulted in brown and dark olive sectors. A deep olive sector from the first whitish sector gave a dark olive and an

almost white sector. A brownish sector from the original single-spore culture resulted in deep olive, grayish, and Dresden brown sectors. One of these grayish sectors gave an albino, a light brown, and a deep olive sector. With the exception of the albino sector, in which color production appeared to be lost completely and permanently, color changes from dark to light or from light to dark did not appear to be due in any case to permanent loss of a color factor.

Twenty-four plates of potato-dextrose agar were inoculated with 221: 1: b, a 60-day old single-spore, dark olive, tube culture (Woodford County). Only 4 culturally distinct strains developed; 2 olives, deep and dark, and 2 browns, Rood's and olive. There were differences in rate of growth, colony characters, and spore production. A few sectors formed in subsequent platings, but this culture was less variable than 224 : 1 : b.

SINGLE-SPORE CULTURES OF SINGLE-SPORE CULTURES

Thirteen single-endoconidium-spore cultures were made from 6 station single-spore cultures and from one Woodford County single-spore culture. In the first plating from 15- to 23-day-old tube cultures no sectors appeared, but in a second plating, when the tube cultures were 27 to 35 days old, many sectors formed. Thirteen more single-spore cultures were made from a 30-day-old tube culture, 224 : 1 : w, (Fig. 2, C) from which 224 : 1 : b, previously described, came. Eight of these cultures appeared alike, grew uniformly, were Dresden brown, and had numerous endoconidia and chlamydospores. The other 5 cultures differed from the 8 and from one another. Sectors developed in 4 of these (Fig. 2 E, F, G, H). When the 13 single-spore cultures of 224 : 1 : w were 28 days old they were plated in triplicate on potato-dextrose agar. These 28-day-old cultures showed greater variation than the 7-day-old cultures. Often, the colonies in 3 plates from one tube were culturally distinct. In the 39 plates, 15 sectors distinctly different from one another and from the original (224 : 1 : w), developed. Two more cultures that produced only chlamydospores developed in 2 sectors. So far, these and the chlamydospore culture isolated from rotting roots have not produced endoconidia, the factor for the production of which appears to have been lost. Tentatively, these cultures may be considered genetically different from the more complete type of organism from which they developed. This series of platings again suggests that the longer cultures grow in tubes the greater will be the number of variants when plated. Single-spore cultures seem to be as unstable as nonmonosporous cultures, although much additional work should be done to establish this point.

ALBINO CULTURE

An albino culture (Fig. 1, H) having both chlamydospores and endoconidia, obtained by subculturing a white sector from one of the station

FIG. 2. Cultures of *Thielaviopsis basicola* after 12 days on potato-dextrose agar. A (224: 1). Isolation from rotting tobacco roots; not a single-spore culture. B (224: 1: v), C (224: 1: w), and D (224: 1: z). Single-spore cultures from A. E (224: 1: w: 8), F (224: 1: w: 9), G (224: 1: w: 10), and H (224: 1: w: 16). Single-spore cultures from the single-spore culture shown in C.

cultures, has developed no color either in the hyphae or in the chlamydospores during 4 years of propagation. Seven single-spore cultures, from this albino, developed 3 culturally distinct colonies on potato-dextrose agar but all were white and had endoconidia and chlamydospores.

SPORE VARIATIONS

The cultures of *Thielaviopsis basicola* showed considerable variation in the number, kinds, and sizes of spores. In some, endoconidia predominated; in others, chlamydospores greatly outnumbered endoconidia. The complete absence of endoconidia in 3 cultures has been mentioned. No culture, having endoconidia only, was found in any of the tubes or plates. The endoconidia varied little in size from culture to culture, but there was considerable variation in the chlamydospores; some were borne in clusters of 3 to many; in others they were terminal and single; and in others single and intercalary. Some cells, which were spherical, hyaline, terminal or intercalary, and either solitary or in chains of three or more, developed in some cultures. These may represent another spore type.

HYPHAL CHARACTERS

In general the microscopic variations of the hyphae, in size and method of branching, were not great, being often as great within a culture as between cultures. One culture, however, developed, in addition to the normal mycelium, dark colored strands that readily broke into sections composed of single cells.

NO PERFECT STAGE OBSERVED

Plates were inoculated with different combinations of single-spore cultures, incubated at 27° C., and grown for some time after the hyphae intermingled, but no sexual fruiting bodies were observed. Many cultures were kept in tubes for a year, but no sexual fruiting bodies developed.

PATHOGENICITY TESTS

Eight single-spore cultures, including the albino and a hyphal-tip chlamydospore culture, differing markedly from one another in cultural characters, were used to inoculate a black-root-rot susceptible strain of White Burley tobacco. The roots of seedling plants were dipped in a water suspension of a 28-day-old agar culture of the organism. The plants were then set in jars of steamed soil in holes in which were placed agar cultures of the organism. An examination of some of the plants at the end of 3 weeks showed typical black root rot, but little or no difference in amount of rotting caused by the various cultures except that the albino seemed to be slightly more pathogenic than the others. After 5 weeks the amount of black root rot was the same for all cultures except the albino, which still seemed slightly more pathogenic than the others.

DISCUSSION

Gilbert (2), and McCormick (4), grew *Thielaviopsis basicola* on artificial media, but neither mentioned variability. The limited tests indicate that *T. basicola* is as unstable in culture as the smuts, Fusarium, Phytophthora, and others.

The cause of cultural variations of imperfect fungi is unknown and will be difficult of solution until more is known of their nuclear constitution. There seems to be some genetic significance to the changes observed, although they occur in what now appears to be a random manner. *Thielaviopsis basicola* may carry at least one and possibly several factors for color, factors for the production of at least 2 and possibly more kinds of spores, as well as factors that determine hyphal types. Some of these factors, such as those for the production of endoconidia and those for color appear to have been completely lost in certain cultures. The mechanism by means of which this suppression or loss of factors occurs is unnkown.

Thielaviopsis basicola, because of its unstable constitution, its many color and other physical characters, and its 2 distinct spore types, should be an ideal organism for studying the genetics of the imperfect fungi when a technic for such studies is finally worked out.

SUMMARY

Eleven cultures of *Thielaviopsis basicola* isolated from rotting roots of White Burley tobacco, differed from one another when grown on potato-dextrose agar. These cultures, after having grown a few weeks in test tubes, formed numerous sectors when transferred to potato-dextrose agar in Petri dishes, possibly due to some kind of segregation during growth in the tubes. Single-endoconidium cultures of the above differed from one another and were also unstable in culture. Single-spore cultures of single-spore cultures were as unstable as the original isolants and the single-spore cultures from which they came.

A culture producing only chlamydospores was isolated from rotting roots of tobacco. Two other chlamydospore cultures developed as sectors and none of these produced endoconidia during 1 or 2 years' propagation.

An albino culture, having both endoconidia and chlamydospores, developed as a sector in one plate. This was subcultured and kept for 4 years, but did not produce any color. Seven single-spore cultures of the albino were white and had both types of spores, although there were 3 culturally distinct types.

Eight single-spore cultures, differing in appearance, showed little difference in pathogenicity.

Sexual fruiting bodies did not develop in old cultures nor in plates containing 2 or more intermingling single-spore cultures from different sources.

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FACTORS AFFECTING EXPERIMENTAL ERROR IN GREENHOUSE POT TESTS WITH SUGAR BEETS[1]

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INTRODUCTION

Despite effort to secure relatively uniform temperature conditions within a greenhouse, it is the common experience that plants within a test series are not given the same temperature exposures. These temperature differences may be large if the heating system consists of vertical radiating coils located on the side walls or ends of the greenhouse. Obviously, the differences are still greater if some of the plants are placed close to the pipes and exposed to direct radiation.

The degree of experimental error caused by such temperature differences has been given little consideration in plant pathological experiments. The results of two experiments designed to ascertain the fluctuation in damping-off effects in a series of pots are illustrative of the magnitude of the error. The temperature records taken with calibrated thermographs at two locations on the plant tables reveal important differences that probably more or less directly influenced the incidence of damping off. Methods are suggested as to ways and means by which the variance assignable to temperature and other positional effects likely to be encountered in greenhouse tests may be determined and removed from consideration.

METHODS

The data presented were obtained in the winter of 1933 in tests to determine the capacity of soil pathogens to cause damping off of sugar-beet seedlings growing in 4-inch pots. In the first series, the plant table was a raised bed with concrete walls about 18 inches high. This bed was filled level full with soil, and the pots stood fully exposed on this solid plant table. In the second series, the pots stood on an ordinary greenhouse bench having 6-inch side walls. A vertical coil of heating pipes was situated along one side and at one end of both the concrete plant table and the board-wall bench. As shown by the photographs (Fig. 1), the raised concrete bed was more exposed to direct radiation than the board-wall bench. Calibrated thermo-

[1] The data presented in this paper were obtained in cooperative investigations by the Division of Sugar Plant Investigations, Bureau of Plant Industry, United States Department of Agriculture, and the Division of Plant Pathology and Botany of the Minnesota Agricultural Experiment Station. Paper No. 1322 of the Journal Series of the Minnesota Experiment Station.

[2] The writer gratefully acknowledges the helpful criticism of Drs. G. H. Coons and E. C. Stakman in the preparation of the manuscript and to Dr. F. R. Immer for aid in the statistical analyses.

graphs were maintained in the center of both sides of each plant table. As shown in figure 1, the Bourdon tube of each instrument was protected from direct radiation of the sun.

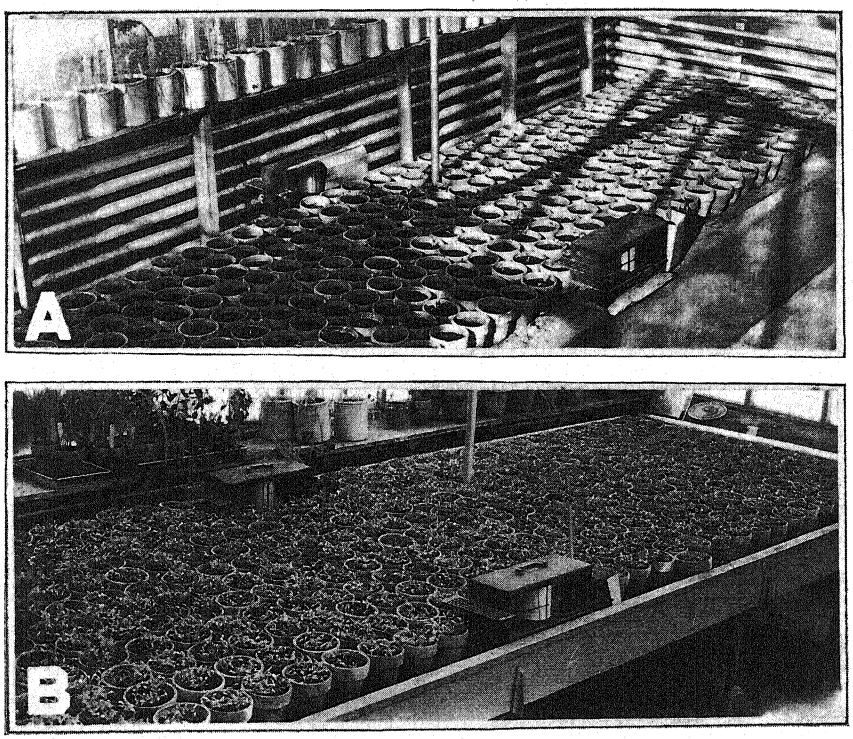


FIG. 1. Plant tables upon which uniformity studies with sugar beets were made. A. Concrete bed. B. Board-wall bench. Note the position of heating pipes and thermographs in each case.

Attempt was made to secure uniform soil conditions in the pots. Non-steamed, thoroughly mixed garden soil, into which a small amount of *Rhizoctonia solani* Kühn from sugar beet had been evenly incorporated, was used. In the first experiment, 400 four-inch pots were arranged in 40 equal rows running across the raised concrete plant table. In the second experiment, 432 four-inch pots were arranged in 36 equal rows running across the board-wall bench. Fifty seed balls of K. W. Normal sugar beets were planted in each pot, and covered to a uniform depth of one inch. Stand counts were made for each pot two weeks after planting. The data were analyzed by Fisher's analysis-of-variance method, the significance of the results being determined from the appropriate tables given by Fisher.[3]

[3] Fisher, R. A. Statistical methods for research workers. Ed. 4, rev. and enl., 307 pp., illus. Oliver and Boyd, Edinburgh and London. 1932.

RESULTS

The analyses of variance of the stand counts for each of the 400 pots on the concrete bed are given in table 1.[4] It is seen that the variance "between columns" with respect to the variance "within columns" is highly signifi-

TABLE 1.—*Analyses of variance of seedling stands in 400 4-inch pots placed on the surface of a concrete bed in the greenhouse*

Variation	Sum of squares	Degrees of freedom	Variance	z
		Between the columns		
Between columns	4,591.70	9	510.18	0.5146[a]
Within columns	71,116.05	390	182.35	
Total	75,707.75	399		
		Between the rows		
Between rows	8,281.15	39	212.34	0.0626[b]
Within rows	67,426.60	360	187.30	
Total	75,707.75	399		

[a] Exceeds 1 per cent point.

[b] Does not reach the 5 per cent point.

cant, the observed z exceeding the value of that statistic at the 1 per cent point. On the other hand, the variance "between rows" as compared with the variance "within rows" does not reach a significant value.

From the data obtained, it was seen that more seedlings emerged in the pots immediately adjacent to the heating pipes. The total for the column (1,838 seedlings) was significantly higher than the total for any other column. The seedling stands of Row 1 (next to the pipes) and Row 40 (farthest from the pipes) were higher (434 and 440 seedlings, respectively) than any of the rows immediately adjoining, but the differences between rows were not significant for the experiment as a whole; hence these variations are interpreted as due to chance.

The data for the second experiment, involving 432 pots in a board-wall bench, were similarly analyzed, and it was found from comparison of the variance "between columns" with that "within columns" that significant differences did not exist (Table 2). In the comparison of the variance "between rows" with the variance "within rows," the difference was highly significant, since the z value exceeded that for the 1 per cent point. Thus,

[4] In the calculation of variances in the experiments, the stand counts of the individual pots were (a) combined as rows running across the plant table to determine the variance "between rows" and (b) the appropriate readings for the individual pots were then considered in their lengthwise alignment on the plant table, to determine the variance "between columns." The individual readings of stand counts of the two experiments are not printed but are available to any one interested.

TABLE 2.—*Analyses of variance of seedling stands in 432 4-inch pots placed on a board-wall bench in the greenhouse*

Variation	Sum of squares	Degrees of freedom	Variance	z
		Between the columns		
Between columns	2,201.14	11	200.10	[a]
Within columns	90,021.64	420	214.34	
Total	92,222.78	431		
		Between the rows		
Between rows	17,612.78	35	503.22	0.4912[b]
Within rows	74,610.00	396	188.41	
Total	92,222.78	431		

[a] Not significant.

[b] Exceeds the 1 per cent point.

with the board-wall bench, the situation as to positional effect was the reverse of that with the concrete bed.

The temperature records as obtained from the recording thermographs, and summarized in table 3 as minimum, maximum, and mean temperatures

TABLE 3.—*Minimum, maximum, and mean temperatures on heated and non-heated sides of the concrete bed and the board-wall bench in the greenhouse*

Temperature	Type of plant table	
	Concrete bed	Board-wall bench
	°F.	°F.
Minimum		
Heated side	71.9	59.4
Non-heated side	64.2	63.8
Difference	7.7	4.4
Maximum		
Heated side	89.0	78.9
Non-heated side	81.1	82.3
Difference	7.9	3.4
Mean		
Heated side	80.4	69.1
Non-heated side	72.6	73.0
Difference	7.8	3.9

for the side of plant tables nearer the heating pipes and the side farther removed, give an indication of at least one factor that was concerned in the variation found. It is seen that, on the average, the side of the concrete bed near the heating pipes was about 8 degrees F. warmer than the side more

removed. In the case of the board-wall bench, the condition was reversed, the mean temperature for the side near the steam coil was, on the average, from 3 to 4 degrees F. lower than the side more removed.

It would seem probable that these temperature differences, in the case of either the concrete bed or board-wall bench, and especially in the case of the former, are sufficiently great to cause differences in response in tests involving soil-borne pathogens.

The analyses made indicate that there is a definite positional influence on each type of plant table. It appears clear that this heterogeneity in temperature exposure, with its attendant effects on drying of the soil, together with the recognized differences in light exposure in the greenhouse, should be considered in designing experiments.

In agronomic field work, following the suggestions of Fisher,[5] a restricted randomized arrangement of plots is utilized that permits assignment of the variance arising from place effects, and its removal from consideration in the comparison of variance to be assigned to treatment with that designated as error.

The data from these 2 uniformity tests may be used to test the value of an experimental design that would permit removal of the variance assignable to position on the plant table. An experimental design suitable for such a test would be the equalized randomized-block arrangement suggested

TABLE 4.—*Analyses of variance of the two hypothetical Latin-square arrangements of the data obtained from the uniformity tests made on a raised concrete bed and a board-wall bench in the greenhouse*

Variation due to	Sum of squares	Degrees of freedom	Variance	z
		Raised concrete bed		
Rows	1,256.20	9		
Columns	4,591.70	9		
Strains	9,536.15	39	244.52	0.1632[a]
Error	60,323.70	342	176.38	
Total	75,707.75	399		
		Board-wall bench		
Rows	12,488.64	11		
Columns	2,201.14	11		
Strains	4,053.03	35	115.80	[b]
Error	73,479.97	374	196.47	
Total	92,222.78	431		

[a] Does not exceed the 5 per cent point.

[b] Variance due to strains less than that due to error; therefore, strains are not significantly different.

[5] *Loc. cit.*

by Student.[6] Taking the data from the test on the concrete bed one may assume that 40 strains of a fungus were to be tested, using 10 replications. In this arrangement, the 40 strains as represented by 10 pots each are placed at random on the bench, the only requirement being that each strain occur but once in each row-group (4 rows) and once in each column. Such an arrangement was devised, and the values obtained in the 2 uniformity tests were taken as appropriate readings.

Table 4 gives the analysis of this hypothetical experiment on the raised concrete bed. It is seen that the variance assignable to strains is not significant. Since the disease incidence among the pots was fairly uniform, the variance originally shown (Table 1) probably was due largely to general differences in the exposures on the bed. This failure of the hypothetical strains to show significant differences is what is to be expected, provided the variance assignable to location is removed.

Similarly, if another hypothetical test of 36 strains, replicated 12 times in accord with the same experimental design, is set up using the data from the pots on the board-wall bench, positional effects may be determined and when removed strains are, likewise, found to be not significant (Table 4).

It is to be noted that in the analysis (Table 2) of the data of the second experiment, a significant difference "between rows" was found; therefore, any systematic replication of pots would have been likely to give a false indication of the situation and cause one to assign a difference, in reality due to position, to the organism that happened to be placed in that row.

SUMMARY AND CONCLUSIONS

In greenhouse tests, attempt is made to establish uniformity of conditions except for the factor under consideration. The uniformity tests reported afford indication of the variations in conditions likely to be encountered and their effects. In one uniformity test the percentage of damping off of sugar beets in the border row of pots on a raised concrete bed was markedly less than that in the adjacent rows of pots. This effect was less marked, or almost absent, in a bench with board walls, because of deflection of direct heat. Obviously, uniformity of environmental conditions would be improved by suitable devices for deflection of direct heat, and, in greenhouse experiments, the possibility of temperature differences needs to be considered. The second experiment showed that, on a board-wall bench, unsuspected environmental factors also operated that resulted in certain rows showing significantly different amounts of damping off. These differences were of such magnitude as to bias judgment had the experiment been set up in a systematic arrangement so that, for example, all inoculations with cer-

[6] Student. Yield trials. *In* Baillière's Encyclopedia of Scientific Agriculture. Vol. 2, pp. 1342–1361. Baillière, Tindall & Cox, London. 1931.

tain pathogens were located in such rows. Since these variations in environmental conditions may occur and may not be fully anticipated, the applicability of certain methods now in use in agronomic field experiments is pointed out. In meeting such problems in the field, experimental designs are used in which the variates are so randomized as to avoid localization and to permit an evaluation of place effects. It is found that the use of a suitable design not only adds to the efficiency of the experiment but also serves to take into account some, at least, of the unsuspected factors that might bias interpretation.

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THE DEMONSTRATION OF PLANT-PARASITIC NEMATODES IN HOST TISSUES

G. H. GODFREY

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The method of demonstratinn, by staining and clearing, of plant parasitic nematodes in the host tissues, described by Steiner (4) and later, with slight modification, by the writer (1), is here modified still further. The basic principle is the reduction of osmic acid by the fats and lipoids of the nematodes, with production of a dark color, as explained in Lee's Vade Mecum (3). While satisfactory for roots and other materials not containing excessive oils and lipoids, the old method was unsatisfactory for green plants because of the dark color produced in the tissues by quick reduction of the osmic acid of the killing fluid, which completely obscured the nematodes.

Attempts were made by various methods to eliminate from the leaves the materials that bring about this effect. Most of them failed because the reagents removed such materials simultaneously from the nematodes. A successful procedure here described finally was adopted.

Materials.—80 per cent acetone in water; Flemming's Strong killing fluid; graduated alcohols, from 15 to 100 per cent; clove oil.

Procedure.—The method, in brief, follows:

(1) Bring some 80 per cent acetone to boiling in a test tube or small Erlenmeyer flask. Since the boiling point of this solution is about 63° C., this may be done by immersing the container in hot (75 to 90° C.) water, or even by allowing some hot tap water to flow over it.

(2) Quickly submerge in the hot acetone solution small pieces of leaves, stems, or galls known or suspected to contain nematodes. The nematodes will be killed promptly without appreciably modifying their internal structure, and the lipoid-extracting process will then commence. Leave the materials in the gradually cooling solution for 3 to 4 hours or until the chlorophyll is removed.

(3) Pour off the acetone and wash the materials in 3 or 4 changes of water.

(4) Immerse the materials in Flemming's Strong killing fluid. This may be done in a Syracuse dish under the binocular in order to observe the depth of coloration of the nematodes and the contrasts obtained.

(5) Wash to remove the killing fluid; dehydrate in alcohol; and infiltrate and clear with clove oil. Clove-oil mounts of the materials may now be made for temporary observation or for photographing.

(6) For permanent mounts, replace the clove oil with Canada balsam.

Practice will indicate the desirable periods of exposure for each of the reagents used. The boiling point of pure acetone is 56.4° C. By diluting this slightly in water, not only is the lipoid-extracting process retarded and less violent, but the boiling point is slightly raised—both, desirable features. The boiling point of 80 per cent acetone, about 63° C., is not

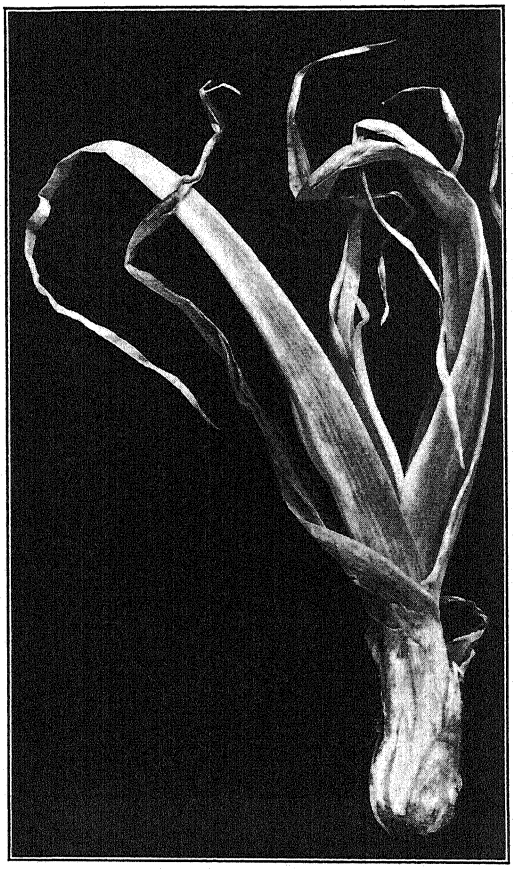


FIG. 1. A garlic plant infested with the stem and bulb nematode, *Anguillulina dipsaci*. The nematodes are abundant in the outer bulb scales and outer leaves, though they are not evident on superficial examination.

too high for satisfactory nematode fixation. The danger of excessive heating is thus eliminated. The extraction, of course, must not be carried too far; otherwise the nematodes will not become colored. The desired point is best reached for demonstration of the nematodes in the tissues by occasionally removing a piece of the material and testing it with Flemming's fluid. As soon as the plant tissues no longer quickly turn dark, the rest

of the materials may safely be removed and subjected to the Flemming's treatment. Usually from 5 to 10 minutes in this solution suffices for demonstrating the nematodes. If they do not show up in this time the extracting process probably has been carried too far.

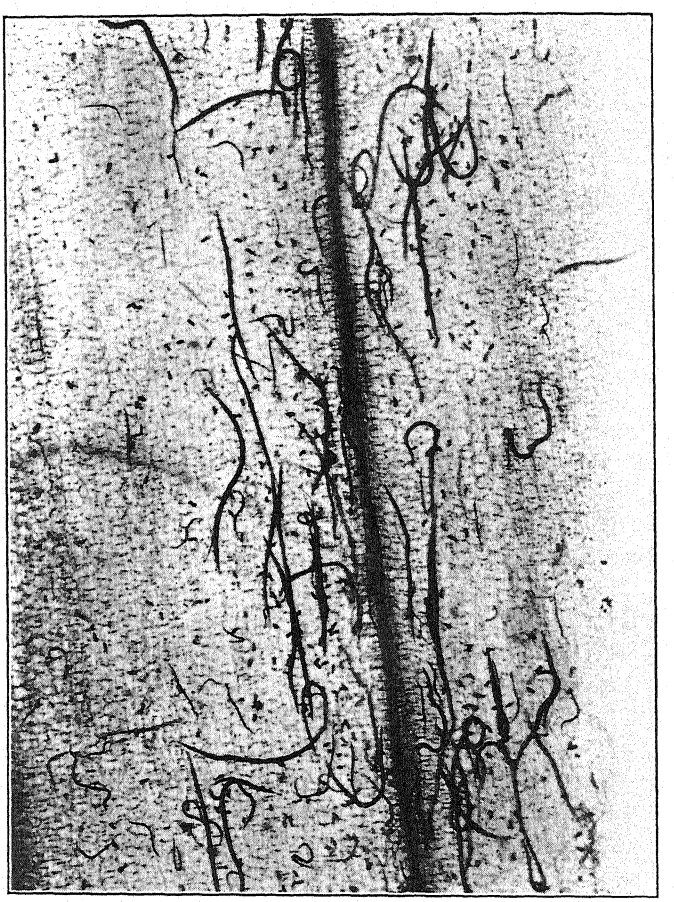


FIG. 2. A small block clipped from the side of a green leaf of a nematode-infested garlic plant, prepared according to the method herein described. Nematodes in all stages of development are clearly differentiated in the leaf tissues. ×16.

Figure 1 is a garlic plant heavily infested in the bulb scales and outer leaves by the stem and bulb nematode, *Anguillulina dipsaci*. In green parts of the leaves the swelling and distortion commonly produced in plants by this nematode were lacking, so nematode invasion was not demonstrable except by dissection. Rectangular blocks of full thickness

were taken from infested bulb scales and green leaves and processed according to the foregoing method, both with and without the acetone treatment. Those without the acetone became quite black and the nematodes were obscured, except for an occasional individual protruding from the cut edge. Figure 2 is a photomicrograph of a green leaf block receiving the full process, as it appears mounted in clove oil in a hollow slide. Eggs, larvae, and adults are very sharply defined, just as they lie in the leaf tissues. A block from the side of a parsley leaflet containing the same nematode is pictured by Godfrey and Scott (2, fig. 2, A). The writer has held such materials for nearly two years without reduced contrast.

With acetone as the killing medium, the writer has been able to control the period of extraction to the point that some of the fats were removed from the nematodes, so that subsequent exposure to Flemming's fluid darkened them only slightly. In this way he has been able to bring out very clearly the internal organs of the nematodes including the very sharply delimited ovaries and testes. Whether or not this method can be adapted to differential staining technique with the usual nematode stains, remains to be determined by further investigation. The block of plant tissue constitutes a convenient medium in which the nematodes can be carried through the various differentiating processes assembled in one piece of convenient size for handling. At any desired stage they can be removed by dissection and given further individual processing.

SUMMARY

The staining of plant-parasitic nematodes within the host tissues that results from treatment with Flemming's killing fluid is due to the reduction of the osmic acid by the fats and lipoids of the nematodes. Frequently the plant tissues become black as well, due to the contained reducing agents of one kind or another, and the nematodes are obscured. This is particularly true with green leaves and stems. This difficulty can be overcome by first treating the blocks of plant materials momentarily with hot, slightly dilute acetone, which kills the nematodes quickly in their natural position in the host tissues; then subjecting them to the extractive influence of the solution for 3 or 4 hours. This removes the chlorophyll and at the same time removes, at least in part, the lipoids and other reducing substances. The specimens can then receive the regular Flemming's treatment, followed by washing, dehydration in alcohols, and clearing, with excellent results in nematode differentiation.

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DIFFUSIBLE NATURE OF THE INHIBITORY AGENT PRODUCED BY FUNGI[1]

J. CEDRIC CARTER[2]

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INTRODUCTION

The growth of specific fungi on certain substrates causes the latter to be altered. Further growth of a fungus on such an altered substrate may be retarded or entirely prevented. A substrate thus affected is said to have become stale. Each fungus produces a characteristic type of growth on a given substrate. When one fungus is grown in association with another, the characteristic type of growth of each may be altered. On a solid substrate the organisms may grow and meet. One mycelium may overgrow the other or it may intertwine with the hyphae of the associating organism. In other associations the organisms may never make contact because of the production of inhibitory substances. Here again the substrate becomes stale, and the organisms are prevented from making contact with each other. This phenomenon of inhibition occurs when 2 or more organisms that grow in association with each other hinder or prevent the expected development of any one or all of the organisms concerned.

Such retardation or prevention of fungus growth and spore germination is discussed by Bisby (1), Brown (4), and Pratt (17). The work of Boyle (2), Brown (5), Fahmy (10), Frost (11), Machacek (14), Porter (15, 16), and Pratt (18) demonstrated some of the characteristics of a medium that had been staled by fungus growth. Broadfoot (3) and Henry (12, 13) studied the phenomenon of inhibition of microorganisms when they grew in association with each other under natural conditions. The work of Endo (7, 8, 9) demonstrated that the reactions of microorganisms that grow in association with each other are influenced by staling products in many associations. The inhibitory effect on the growth of one organism by the products of growth of another associating organism was utilized by Coons and Strong (6) to differentiate species of Fusarium. This effect was used by Stevens and Ragle (19) to differentiate races of Sclerotium.

DISCUSSION

The observations and results of many investigators as previously indicated show that many different microorganisms exhibit the phenomenon of inhibition when in association with other microorganisms. In the present studies several of the more common parasitic and saprophytic microor-

[1] Submitted in partial fulfillment for the Ph.D. degree.

[2] The writer gratefully acknowledges the counsel given by Dr. C. L. Porter.

ganisms were grown in association with one another in order to determine the relative degrees of inhibition that would be demonstrated by different combinations of microorganisms. *Helminthosporium sativum* was inhibited consistently by a bacterium that was listed as 9a2. These two organisms, when grown in association on potato-dextrose agar, inhibited the growth of one another at distances of 10 to 15 mm.

The following procedure was used to obtain inhibition of growth of these organisms. Sterile Petri dishes were supplied with approximately 20 cc. of sterile potato dextrose agar. *Helminthosporium sativum* was planted in the center of the solidified agar. After the fungus had grown for 24 to 48 hours the bacterium 9a2 was planted in a complete circle around the fungus and as near the border of the Petri dish as was convenient. Within four to six days inhibition of the growth of both organisms was evident. This was shown by a 10 to 15 mm. sterile region between the two organisms. This sterile agar is considered to be stale because it has the ability to inhibit the further growth of *H. sativum*.

A portion of the stale agar was collected, placed in test tubes, and autoclaved at 15 pounds' pressure for 20 minutes. It was then poured into sterile Petri dishes and allowed to solidify; then, it was cut into rectangular sections. Each section was placed on agar a few mm. from the advancing circumference of *Helminthosporium sativum*. In like manner fresh agar sections, and stale agar sections that were not autoclaved, were placed before the growing circumference of *H. sativum*. These constituted checks. Growth of *H. sativum* was inhibited in a region about the sterilized and non-sterilized sections, but was not retarded by the fresh agar sections. The stale agar after it was subjected to autoclaving at 15 pounds for 20 minutes was as active in preventing the further growth of *H. sativum* as was the stale agar that was not autoclaved. It is evident that the inhibitory agent in the stale agar not only prevented the fungus from growing over the stale agar, but diffused out of the stale agar sections into the fresh agar; thus, preventing the fungus from growing to the stale agar sections.

After finding that the inhibitory agent diffused from the stale into fresh agar, an attempt was made to cause the inhibitory agent to diffuse from the stale agar into water.

The agar staled by *Helminthosporium sativum* and bacterium 9a2 was placed in 100 cc. quantities of sterile distilled water in 250-cc. flasks. All aseptic precautions were followed in transferring the agar. The stale agar was removed from the water after 48 hours. This water containing the diffusible ingredients of the agar may be designated as "stale water." The stale water was substituted for distilled water in preparing potato-dextrose agar, which may be designated as stale-water agar. The type and rate of growth of *H. sativum* on this medium was compared with that on potato-dextrose agar after 7 days' growth.

The average diameter in many tests of colonies of *Helminthosporium sativum* on normal potato-dextrose agar in 7 days was 80 mm., while that on stale-water agar was only 20 mm., or an average difference of 60 mm. The maximum difference in growth on the 2 agars at the end of 7 days was 81 mm. There was excessive spore production on the stale-water agar.

The agar that contained the diffusible products from 9a2 and from *Helminthosporium sativum* was tested for potency of the inhibitory agent. The agar from the 9a2 caused a greater inhibition of the growth of *H. sativum* than that from the *H. sativum*. However, the agar from the associated growth of 9a2 and *H. sativum* caused a greater inhibition of the further growth of *H. sativum* than the agar from the 9a2 alone. Thus, the 9a2 is more important in the production of the inhibitory products than *H. sativum*.

Helminthosporium inaequalis, when grown on stale-water agar, was inhibited like *H. sativum*. However, *Fusarium conglutinans* was not inhibited noticeably when grown on stale-water agar.

CONCLUSIONS

1. Certain combinations of microorganisms exhibit a specific antagonistic action toward one another.

2. Stale agar, produced by the growth association of *Helminthosporium sativum* and bacterium 9a2, will inhibit the further growth of *H. sativum*.

3. *H. sativum,* growing on potato-dextrose agar, will not grow in contact with stale agar that has been placed several millimeters beyond the growing fungus; thus showing that the inhibitory agent in the stale agar diffuses into the fresh agar.

4. The inhibitory agent present in the stale agar is thermostable.

5. The inhibitory agent will diffuse from stale agar into water and will retain its inhibitory properties after sterilization.

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CULTURAL STUDIES ON THE VIRUS OF TOBACCO MOSAIC

FRANK H. JOHNSON

(Accepted for publication February 18, 1935)

Of the many studies that have been made on tobacco mosaic, relatively few have dealt with attempts to culture the virus *in vitro*. These studies are characterized either by negative or equivocal results (2, 3, 5, 6, 7, 8). In the present state of our knowledge, however, it is not possible to say definitely whether the virus is or is not of the nature of a living principle, or whether it can or cannot be cultured *in vitro*. The purpose of this note is to communicate some results of attempts made at Duke University, 1931 and 1932, to culture the virus.

The healthy tobacco plant was regarded as the most likely source of suitable constituents for a culture medium; and wherever possible sterilization of the medium by autoclaving was avoided in order not to add to the changes of the natural constituents of the plant during the preparation of the medium. In all cases the absence of the virus in noninoculated media was made certain by inoculating plants. A given amount of medium (usually 9.9 cc. in a test tube) was inoculated with a given amount of virus (usually 0.1 cc. of sap from mosaic plants, rendered bacteria-free by passing through a Berkefeld "W" filter) and incubated 7 days at 30° C. The infectivity of the culture was then tested by inoculating tobacco plants, and at the same time a sub-plant was made into another tube of the same medium. Successive sub-plants were made in this manner, testing the infectivity at each stage through dilutions of 1:1,000,000. The original inoculum was never found to be infectious in a dilution of more than 1:100, and sometimes not even that, probably as a result of the preliminary filtration (4). Both aerobic and anaerobic cultures were maintained, the latter by means of a layer of toluol, which, itself, evidently exerts no harmful action on the virus (1).

A summary account of the culture media prepared is a follows:

First series, from the sap of healthy plants. Fresh leaves were ground, the sap expressed under 10,000 pounds' pressure, and centrifuged. The supernatant liquid was extracted successively with benzol, ether, chloroform, and petroleum ether, retaining after each extraction a portion to be used as culture medium following sterilization by filtration. Cultures inoculated with portions extracted with benzol alone gave no indication of an increase in potency for infection, but in those extracted with both benzol and ether, as well as those extracted further with chloroform and petroleum ether, infections resulted from the 1:1,000,000 dilution, although the inoculum for the cultures was not infectious when diluted 1:100. When

the whole experiment was repeated, the results were not duplicated, for there was no such indication of an increase in potency for infection.

A solution of some of the proteins contained in the sap was prepared by completely saturating the sap with ammonium sulphate at room temperature, and filtering and resuspending the precipitate in a small amount of distilled water. This was dialyzed through a collodion membrane in running water, diluted with a phthalate buffer at a pH of 5.4 to 5.6, and filtered through a Berkefeld "W" filter. Using the filtrate as a culture medium, there was no evidence of an increase in infective power.

Second series, from fresh, unground, healthy leaves. The leaves were extracted thoroughly with 95 per cent alcohol, washed with anhydrous ether, ground to a powder in a mortar, and desiccated over calcium chloride. For a culture medium, 2 per cent by weight was added to a phthalate buffer solution at the above pH, allowed to stand for a while at 30° C., and filtered through a Berkefeld "W" filter. There was no increase in infective power of the virus when implanted in this medium.

In a third series of experiments, fresh, healthy leaves were surface-sterilized by Hexylresorcinol solution (S.T. 37) and by a mercuric chloride solution, washed in sterile distilled water and placed in a sterile cloth that was then lowered into a container of liquid air for an interval of 15 minutes. The frozen leaves were ground, as asceptically as possible in a sterile mortar and transferred to flasks containing sterile distilled water. These were used as culture flasks, with negative results.

The benzol, ether, and cholorform extractives of the healthy plant sap, as well as the alcohol extractives of the leaves, after removal of the solvents and autoclaving in phthalate buffer solution, exerted no apparent action on the potency for infection of the virus contained in the filtered sap from mosaic plants.

It would hardly be worth while to list the minor variations of the general procedures mentioned above. A total of over a thousand plants were inoculated in testing the infective power of the various "cultures," without getting any evidence of an increase in infective power except in the one instance cited. As Olitsky has pointed out (6), the apparent increases in potency for infection that are found in cultivation experiments are difficult to interpret. The increase that appeared in the present study was not duplicated in a repetition of the experiment.

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PHYTOPATHOLOGICAL NOTES

An Effective and Easily Applied Method of Inoculating Seed Barley with Covered Smut.—Progress in a study of the physiologic forms of *Ustilago hordei* (Pers.) K. and S. and in the selection and breeding of resistant barleys has been seriously retarded because no effective and easily applied method of seed inoculation has been known. Investigators repeatedly have failed to obtain high percentages of covered smut (*U. hordei*) in plants from seed blackened with spores. Previously it has been necessary to remove the seed hulls before inoculation in order to obtain satisfactory smutting.

Results of the past 2 years have shown the frequent presence of *Ustilago hordei* mycelium in or on the pericarps of seed from smutted crops that had been thrashed and stored under usual farm conditions. This may explain the failures to get smut by dusting spores on the seed surface followed by sowing the seed soon thereafter, and the failures to obtain control of covered smut in farm-stored barley through seed dusting with copper carbonate. Studies on seed inoculation, therefore, were conducted to place inoculum under the hulls of barley kernels. Numerous attempts resulted in the discovery of a relatively easily applied and highly effective method of covered-smut production. This method, as used in a study of physiologic forms of *U. hordei,* is as follows: Six-gram lots of seed are placed in $\frac{1}{8}$ in. x 3 in. shell vials. A spore suspension, consisting of 1 gram of spores added to a liter of water and thoroughly shaken, is poured into the vials until the fluid rises about $\frac{3}{4}$ in. above the seed. The seed is vigorously shaken in this suspension for $\frac{1}{2}$ minute then allowed to soak 15 minutes. The suspension then is decanted and the vials are inverted on clean pieces of blotting paper to absorb all free water. Next, the vials of moistened inoculated seed are packed in a tightly covered tin box ($5\frac{1}{2}$ in. high, 12 in. wide and 17 in. long) floored with a moistened blotter and incubated for 24 hours at 20° C. Lastly the seed is transferred to small envelopes, crimped to remain wide open, where it is left 2 or 3 days or until thoroughly dry. It is then ready for sowing. Through the cooperation of the New York (Cornell) Agricultural Experiment Station, field plantings of spring barley inoculated by this spore-suspension method were made at Ithaca, New York, in 1934 and again in 1935, and up to 70 per cent of smutted heads were obtained. Similar results have been obtained at Statesville, North Carolina, and at Rosslyn, Virginia, with winter barleys. The results at Statesville were obtained through the cooperation of the North Carolina Agricultural Experiment Station and the Division of Forage Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture. The method approaches a combination of the simplicity of dusting seed with spores and

the effectiveness of inoculating dehulled seed with dry spores. Because it is a wet method, spores do not fly about, and, therefore, it is ideally adapted for use in studies of physiologic forms. In this method, much less inoculum is needed than in the dusting method of inoculation, and, as a result, there is a consequent economy in time and greenhouse space necessary to increase small collections. To date, the method has not been injurious to seed germination. More recent studies have shown that the effectiveness of the new method may be further increased by first slightly sprouting the seed, then thoroughly drying it before proceeding with the method of inoculation described above. It is important to note, however, that the seed should be sprouted only to the extent that the radicle barely breaks through the seed-coat.—V. F. TAPKE, Bureau of Plant Industry, Washington, D. C.

The Transmission of Ceratostomella ulmi through Root Grafts.—During investigation and control work on the Dutch elm disease, cases have been observed in which transmission had undoubtedly been accomplished through root grafts between a tree supposedly inoculated in the usual manner and a nearby tree.

Root grafts between elm trees are common, especially in crowded stands. Whenever two roots cross, growth pressure becomes sufficient, as the roots increase in diameter, to flatten them at the point of contact until a complete union results. Excavations of roots of nearly a hundred pairs of elm trees show that nearly all trees over 8 inches in diameter are connected by root grafts if the stems are 7 feet or less apart. The larger the trees the greater the distance at which they will graft. Grafts have been found between trees 17 feet apart, and groups of 3 to 5 trees have been found grafted into a continuous system.

A complete vascular union in root grafts is evidenced by 3 kinds of observations: (1) Roots will live at least 3 years from a graft outwards after the death of that portion between the graft and the parent; (2) dyes can be passed readily from a root of one tree into roots of another through a graft; and (3) the vessels discolored by *Ceratostomella ulmi* (Schwarz) Buisman can be traced through grafts (Fig. 1).

During the spring and summer of 1935 12 trees showing the Dutch elm disease were found grafted to the roots of the stumps of trees known to have been diseased and removed in the regular control program of 1934. The discoloration, from which *Ceratostomella ulmi* was isolated, was traced in each case from the stump, through the grafted roots, and into the stem of the adjacent tree. In 5 of these cases the discoloration in the tree was limited to the grafted roots, collar, and lower stem but absent from the crown. Thus the fungus quite evidently had not entered through the crown, as it does ordinarily, but had entered through the grafted roots. The

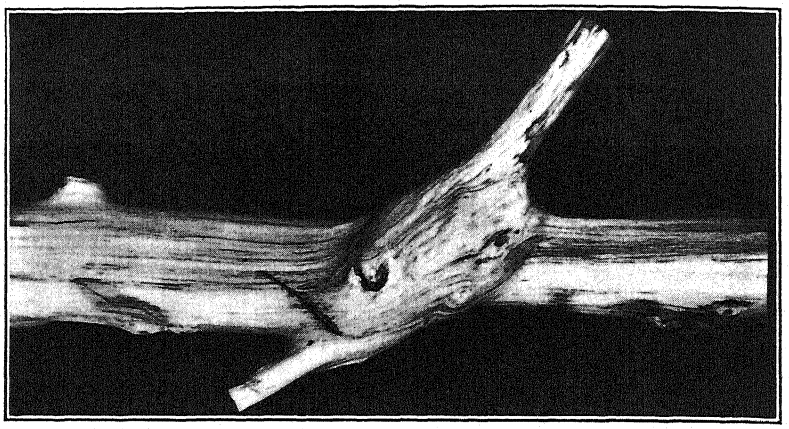


FIG. 1. Roots of two elm trees grafted together. Bark removed to show streaks caused by *Ceratostomella ulmi* passing from one root into the other through the graft.

discoloration was too general in the other 7 trees to be sure that they were not inoculated in the crowns also. However, regardless of whether they were inoculated also in the crowns, the fungus had passed through the grafts.

Streaks from which *Cerastostomella ulmi* was isolated, were traced through root grafts between 5 pairs of stumps removed in the 1934 control program. Nothing is known of the discoloration in these trees at the time of felling to indicate the points of inoculation, but it is possible that *C. ulmi* could have been transmitted to one tree of each pair through the grafted roots and that each would become diseased, even had there been no crown inoculations.—A. F. VERRALL AND T. W. GRAHAM.[1]

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THE EFFECT OF LIGHT INTENSITY AND TEMPERATURE ON INFECTION OF HOPE WHEAT BY PUCCINIA GRAMINIS TRITICI[1]

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(Accepted for publication February 26, 1935)

There are many puzzling discrepancies in the degree of infection caused by *Puccinia graminis* on cereals in different developmental stages. Seedlings of Hope wheat are susceptible to *Puccinia graminis tritici* form 21 when inoculated artificially in the greenhouse. In the field throughout the United States, however, previous to 1933 Hope seldom had more than a trace of stem rust; and whenever rust did appear there was evidence of considerable resistance on the part of the host.

Stakman (30) and Stakman and Piemeisel (33) early observed that cereals and wild grasses varied greatly in their susceptibility to stem rust; some apparently were extremely susceptible when young and much less susceptible when older, others appeared to be more susceptible as they grew older, and many were susceptible in the greenhouse but not severely or universally rusted in the field. Stakman and Piemeisel (33) observed no cases of rust immunity due to age of host, although the rust often was distinctly more virulent on younger or older plants, depending on the peculiarity of the grasses. Levine (21) was of the opinion, from observational evidence, that susceptibility of plants depends to a considerable extent on their stage

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This work was started as a cooperative project of the Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture and the Minnesota Agricultural Experiment Station.

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of development, susceptibility being greater in the seedling and heading stages and less in the jointing stage, so that some varieties appear to be susceptible as seedlings but resistant as they mature in the field. In some wheat varieties such field resistance has been merely an escape from rust by reason of early ripening. Other varieties have appeared resistant in the field because physiologic forms to which their seedlings are susceptible have not been present or have not been abundant enough to cause wide-spread infection. Still others are rust resistant in the field because their stomata remain closed at critical periods for infection and exclude the rust germ tubes. At first it was believed that Hope wheat had a functional resistance by reason of its stomatal behavior (18), particularly since Abbott (1, 2) reported Hope completely susceptible to stem rust in the field in Peru. In 1931 and 1932, however, stomatal movements in this variety grown at St. Paul, Minnesota, were such that stem rust germ tubes might enter the host easily; but even so there was merely a trace of rust in the plots supplied with abundant inoculum of *P. graminis tritici* 21.

Other workers have advanced the theory of mature plant resistance, implying that as the host plant grows older, it acquires more resistance to rust than it had in its seedling stage. Goulden, Newton, and Brown (16) tested the reactions of twelve wheat varieties and two emmers at two stages of maturity, the seedling stage and the after-heading stage, to a number of physiologic forms of *Puccinia graminis tritici.* They concluded that Acme, Hope, Pentad, and H–44–24 developed marked mature-plant resistance, whereas Garnet, Marquis, and Quality did not, for the rust reactions of these three in the after-heading stages corresponded very closely with their reactions as seedlings. The authors state that the reading of reactions was difficult because there was extreme variation on different plants; consequently the readings were made on the basis of pustule size, not so much attention being paid to chlorosis and necrosis.

Harrington and Smith (17), on the other hand, studied rust reactions in seedlings and in headed plants of Marquillo, Marquis, Iumillo, and Vernal emmer. They found a strong relationship between the seedling reaction and the after-heading reaction, although the percentages of infection after heading often were low as compared with those in seedlings.

Neatby (24), in his study of Marquillo × Reward, Garnet × Marquillo, and Garnet × Double Cross, found no evidence of mature plant resistance but indications that "the inheritance of the field reaction to stem rust, as determined by percentage infection, in these crosses is mainly if not entirely controlled by the factors which govern the inheritance of the seedling reaction to form 21 in the greenhouse, as determined by pustule type."

Similar investigations have been made with *Puccinia triticina.* Johnston and Melchers (20) studied the relation of leaf rust infection to age of wheat

plants and found that a number of varieties that were susceptible as seedlings became highly resistant to *P. triticina* form 9 at heading time. The greatest resistance appeared in the uppermost leaves, so that the infected flag leaf often was flecked while the lower leaves were covered with numerous uredia.

Scheibe (28) reported that the seedling resistance to *P. triticina* characterized by a type 1 or type 2 infection changed to an increased resistance as the individuals grew older and that the higher-inserted leaves were more resistant than the lower-inserted ones. But the varieties with extreme susceptibility and a type 4 reaction and those with extremely high resistance and a type 0 reaction retained their characteristic reactions throughout all growth stages. The increase in resistance in the varieties with low resistance in the seedling stage seems associated with the photosynthetic processes. A decline in photosynthetic activity seems to induce a more rapid development of the telial stage of the rust.

The equatorial countries offer opportunities for further observations on stem-rust infection. Although certain varieties of wheat are resistant to stem rust in the field and even in the greenhouse during their later developmental stages in Canada and the United States, these same varieties are completely susceptible when grown in Peru, South America, and Kenya Colony, East Africa. Abbott (1, 2) has reported that wheat varieties, resistant to stem rust in the fields of northern United States and Canada, are severely rusted in the experimental fields at Lima, Peru. Abbott's plantings were made in June and August, during the winter season when there are heavy fogs and mists on the Peruvian coast. One hundred per cent of Khapli emmer and Hope wheat plants were rusted. Pustules appeared on all plant parts and, although relatively small, often were so numerous on Khapli culms as to coalesce and completely girdle the stem. Several possible explanations have been offered for such differences in rust infection of older plants. Abbott himself (1, 2) suggested that a new and virulent physiologic form of *P. graminis tritici* occurred in Peru, a form similar to form 15, except for its severe attack on Khapli emmer. Some workers in the United States have intimated that there may be strains of host varieties as well as strains of the parasite. However, the seed of Hope used by Abbott was sent to South America from Minnesota, and seed of the same lot produced plants that resisted rust in the United States. It also was suggested that under the environmental conditions described by Abbott the stomatal movements of the varieties in question might permit the fungus to enter the host tissues easily, whereas in the United States the environment often affected stomatal movements of these varieties to such an extent as to exclude the parasite. Altitude also has been considered a factor affecting the rust infection of wheats grown in the equatorial regions, for Abbott (3) noticed

a gradual decrease in stem-rust infection at elevations greater than 8,000 or 9,000 feet.

G. J. L. Burton of Kenya County, East Africa, in correspondence with E. C. Stakman, expressed the belief that altitude probably is a less important factor in rust resistance or susceptibility than people are inclined to believe, its principal rôle being to modify climate sufficiently to enable wheat to grow at the equator. Burton also suggested that photosynthesis, length of day, and intensity of sunlight may be associated in some way with the "failure of certain wheats to show mature plant resistance to stem rust under equatorial conditions" in Kenya Colony. During the "long rains" season from March through May the rusts are severe, but there is little rust during the "short rains" season of November and December.

Gassner (8), on the basis of his experimental work in Uruguay, reached conclusions entirely different from those of most of the preceding workers. His experiments were made in the field, but were so planned and the plantings so arranged as to eliminate as far as possible inequalities of external factors, particularly climate. There was a marked increase in susceptibility to *Puccinia graminis* and *P. coronifera* as the age of the wheat and oats plants increased, the plants that had not reached the shooting stages often having less infection than the older plants, so that there appeared to be a period of "youth resistance" rather than a mature plant resistance. With respect to *P. triticina,* on the contrary, the wheat varieties were markedly susceptible in their early growth stages, but developed a more or less decided resistance as they reached the shooting and flowering stages. Older plants were less resistant than those in the intermediate stages. Gassner and Kirchhoff (9) repeated and amplified Gassner's South American experiments to determine the applicability of the earlier results to conditions in Germany. Although the climate in Germany did not favor the continual seeding experiments, their results were in accord with those secured in South America.

As concerns experience in the United States, there seems little evidence that seedlings are really more resistant to *Puccinia graminis* than older plants. The senior writer's own observations and those of several other observers indicate that seedling plants often become heavily infected when there is sufficient inoculum. The senior writer, for example, has repeatedly seen wheat seedlings very heavily infected in her own field plots. The uredia were entirely normal in appearance, without any indication whatever that the plants had "juvenile resistance." Furthermore, large, erumpent, very healthy uredia often appear on seedlings of wheat in the fall in the Southern States and in the early summer in the Northern States. In the fall of 1934, rust was fairly abundant on volunteer seedlings of oats and wheat in Minnesota. It seems likely that seedlings often escape infection because of relative paucity of inoculum at the time they are growing. Long

experience in culturing stem rust in the greenhouse at St. Paul, Minnesota, certainly does not lend support to the idea of "juvenile resistance." On the other hand, there is abundant evidence that some varieties of wheat tend to become more resistant with age. Varieties resistant to stem rust in the seedling stage in the greenhouse are resistant in the field also, although the converse is not always true. These facts lead the writers to the conclusion that seedlings of the small grains are as susceptible as older plants, or, in the case of some varieties, even more so.

Hope wheat has been resistant to stem rust in the field throughout the Mississippi Valley of North America since 1926, when it was first selected from the Marquis × Yaroslav emmer cross. It seldom had more than a trace of stem rust until 1933, although it had been grown under conditions of artificial stem-rust epidemics for several years. Its field resistance was so marked that plant breeders, using Hope or H–44–24 as rust-resistant parents, have relied upon the field reactions and the "mature plant resistance" in breeding work (6, 13, 14, 15, 16). Clark and Humphrey (6) considered the near-immune reaction of Hope as a new character inherently distinct from resistance or susceptibility and found it to be inherited as a dominant character.

The necessity of determining the nature of resistance to stem rust in Hope wheat is apparent, especially if Hope is to be used to a considerable extent in breeding for rust resistance. If the maturing plants of Hope continue to have a marked field resistance to stem rust over a period of years in the United States it must be determined what factors bring about the change from the complete susceptibility of seedlings to some of the rust forms to the great resistance to all rust forms in the older plants. It also is necessary to explain more fully the rust resistance of Hope in the northern hemisphere and its susceptibility in the southern hemisphere. Is it likely that in the future Hope may be susceptible to rust in the United States?

The present study was made for the purpose of determining as definitely as possible the basis for the marked rust resistance in the field of maturing plants of Hope wheat.

FIELD OBSERVATIONS

In 1933 there was more stem rust on Hope wheat grown in the rust nursery plots at University Farm, St. Paul, Minnesota, than ever had been observed before. Some of the plantings had as much as 35 per cent stem rust, the infection types being recorded as varying from R (resistant) to S (susceptible). In every case the large normal pustules, recorded as S, were on the lowest internodes or just above the nodal swellings of the central internodes, the tissues with the greatest free water content and the most shaded and protected parts of the plants in the field. A little higher on the culm,

or on the basal parts of the central internodes were pustules with heavy spore masses but with the surrounding host tissues blackened or purpled with anthocyanin. Still higher on the leaf sheath the pustules were smaller and the pigmentation greater, and often the leaf-sheath portion an inch or two below the blade was covered with subepidermal pustules or mycelial infections, while those host tissues were soon dried or scalded. On the peduncle and awns the pustules varied from minute but normal pustules on the greenest and most succulent parts to hypersensitive flecks, with the latter type of infection predominating. Short young volunteer culms that were green and vigorous when the rest of the plants were ripenings were completely susceptible to rust, with very little chlorosis or pigmentation.

During the growing season of 1933 at St. Paul there was an abundance of sunlight and very few cloudy days. Temperatures were extremely high during the last half of June and moderately high during July. All wheats were exposed to drying winds, which, with the heat, hastened ripening. Despite the weather there was a heavy epidemic of stem rust and ample opportunity to study evidences of resistance in the field.

Other varieties in the plots were examined to determine whether there was similar rust infection in a number of wheats or if the phenomenon was peculiar to Hope. There was no evidence of hypersensitiveness, pigmentation, or discoloration around pustules on Marquis, Haynes Bluestem, Reliance, Reward, Ceres, Kota, Supreme, Progress, Marquillo, Webster, Mindum, or Nodak. There were some evidences of discoloration, pigmentation, and subepidermal pustules on Pentad and on Kubanka. On Acme, H–44–24, H–35, and on Khapli and Vernal emmers, the reactions were similar to those on Hope. There were all variations of infection, and certain of the host tissues seemed more sensitive to drying and scalding, as was the case in Hope. When various hybrids were examined without immediate knowledge of their parentage, it was observed that some reacted like Hope, while others were without any signs of discoloration or pigmentation. In every case of a hybrid with reaction similar to that of Hope it was found that Hope, H–44, H–42, or H–35 had entered into the cross. Not all crosses examined containing one of these four varieties reacted thus, though most of them did.

On culms of *Hordeum jubatum,* collected July 13, 1933,[4] at Ben Lake, Pope County, Minnesota, there were indications of similar reaction to stem rust. Anthocyanins were distributed through most of the culm tissues, surrounding the normal rust pustules on the lower parts of the leaf sheaths and spreading about the hypersensitive and necrotic spots on the peduncles and the upper leaf sheaths. Apparently in Minnesota in 1933 such field reactions to stem rust were not confined to Hope wheat.

[4] Collected by E. C. Stakman, J. J. Christensen, L. W. Melander, and L. M. Stahler.

The reactions of Hope wheat in all stages of development, from seedlings to maturing plants, to physiologic form 21 of *Puccinia graminis tritici* were studied in the greenhouse and field to determine if the field resistance of Hope might be an escape from rust infection, the result of the interplay of environmental factors, or a true resistance.

THE RUST SUSCEPTIBILITY OF YOUNG HOPE PLANTS

Under ordinary greenhouse conditions *P. graminis tritici* 21 always produced type 4 infection on Hope seedlings; and the plants continued to be completely susceptible (Fig. 1) until they were six or seven weeks

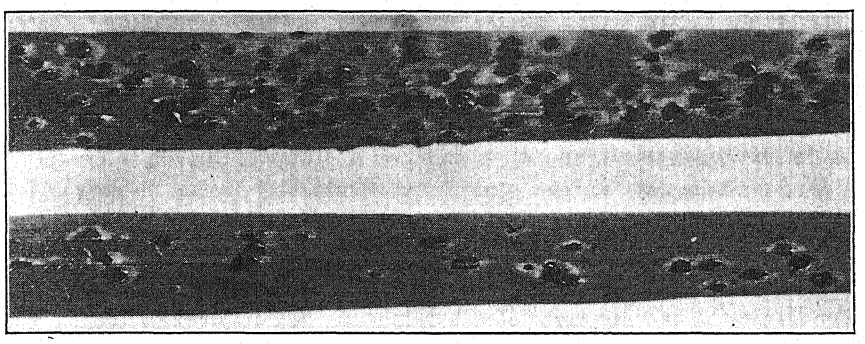


FIG. 1. Leaf blade of Hope wheat 6 weeks old rusted by *Puccinia graminis tritici* 21. The reaction varied from 4 to 4++ with chlorosis.

old, when they had produced five or six succulent green leaves and often were stooling. Uredia were numerous and the fungus sporulated abundantly. Occasionally, the host tissues surrounding an infection were slightly chlorotic, but in no case was there indication of the pigmentation that occurred on Hope in the field and that so often appeared in other hosts resistant to stem rust.

THE RUST REACTIONS OF HOPE IN THE JOINTING, BOOT, AND HEADING STAGES

Older plants, in the pre-boot, the early-boot, or heading stages, were inoculated repeatedly but with very unsatisfactory results, although particular attention was paid to the technic used. Several different methods of applying inoculum were tried, and every effort was made to provide ideal environment for infection. The inoculum was viable and caused severe infection on wheat seedlings inoculated as checks. Temperature varied from 18° to 22° C., soil moisture was sufficient, and the atmosphere remained saturated throughout the moist-chamber period in the greenhouse or for several hours in the field. In many instances inoculations were timed so that the stomata would be open a few hours after the

inoculum was applied, at the time when the greatest percentage of spores presumably had germinated. Inoculum was brushed or shaken over the moistened plants, applied with a flattened needle, or rubbed on with moistened fingers. In the greenhouse the inoculated plants were so placed as to obtain all the sunlight available, especially when experiments were made during the winter months of December, January, or February, the time during which stormy and cloudy weather with the lowest light intensities and the shortest day lengths of the year prevails at St. Paul. Plants were inoculated in the field at different times during the day and evening, and an attempt was made to furnish a saturated atmosphere for a short time after inoculation by enclosing the plants in cloth cages or by wrapping moist cotton loosely around the inoculated culms.

In spite of all technical precautions the older plants seldom rusted. Often there was no sign of infection, especially if the peduncles or the awns had been inoculated. Occasionally, two weeks or more after inoculation in the greenhouse, single pustules or a group of uredia appeared on the leaf sheaths, just at the juncture of blade and sheath or at the base of the sheath close to the node. Such uredia were normal in appearance but never sporulated so abundantly as did the uredia on young Hope plants. In the field in 1932 not even a trace of rust appeared when the older Hope plants were inoculated by brushing or rubbing the urediospores on moistened plants.

In some cases when inoculations were made in the greenhouse chlorotic areas appeared on the culms, peduncles, and awns, but at the time they did not seem to resemble the flecking caused by the rust when the host it attacks is highly resistant to or immune from rust. They seemed more like scalded tissues than rust-infection centers, although later work and the field observations of the following year (1933) indicated that they were infection centers arrested in their normal development. Sometimes the leaf blades seemed entirely scalded after being inoculated and incubated in glass moist chambers exposed to bright sunlight.

The failure to secure more than a trace of rust infection on older plants was difficult to explain. The complete lack of symptoms on so many of the older plants may have resulted from the inhibition of spore germination or germ-tube growth by some toxic substances present in the older plants but not the younger ones. On the other hand, the production of a few small uredia on the most succulent plant parts seemed to belie the acquisition of a protoplasmic resistance to rust by older plants, or at least to raise doubt as to whether certain tissues remained susceptible to rust while young and succulent but suddenly acquired a rust resistance as they approached maturity. To determine the rust reaction of the very young tissues of the older plants, a suspension of rust inoculum was

forced into the leaf rolls and boots of older plants by means of a hypodermic syringe. In addition, experiments were made on the effect of plant extracts on spore germination.

EFFECT OF PLANT EXTRACTS ON GERMINATION OF UREDIOSPORES

The possible presence of a toxic substance in the sap of older plants was investigated by extracting the sap from culms of older plants of Hope wheat growing in the field. The extract was filtered through a Berkfeld filter, prepared in three different dilutions, and the effect of the filtrate on urediospore germination of *P. graminis tritici* 21 studied and compared with the effect of a similar filtrate obtained from culms of the rust-susceptible variety Marquis. The percentage of spores that germinated in the filtrates of different concentration was not so high as for spores in distilled water. However, urediospores germinated as well in extracts of Hope wheat as in those from Marquis, regardless of concentration of filtrate; hence there seemed to be no inhibiting substance in Hope that would prevent the germination of rust spores.

Furthermore, some of the material inoculated by means of the hypoder-

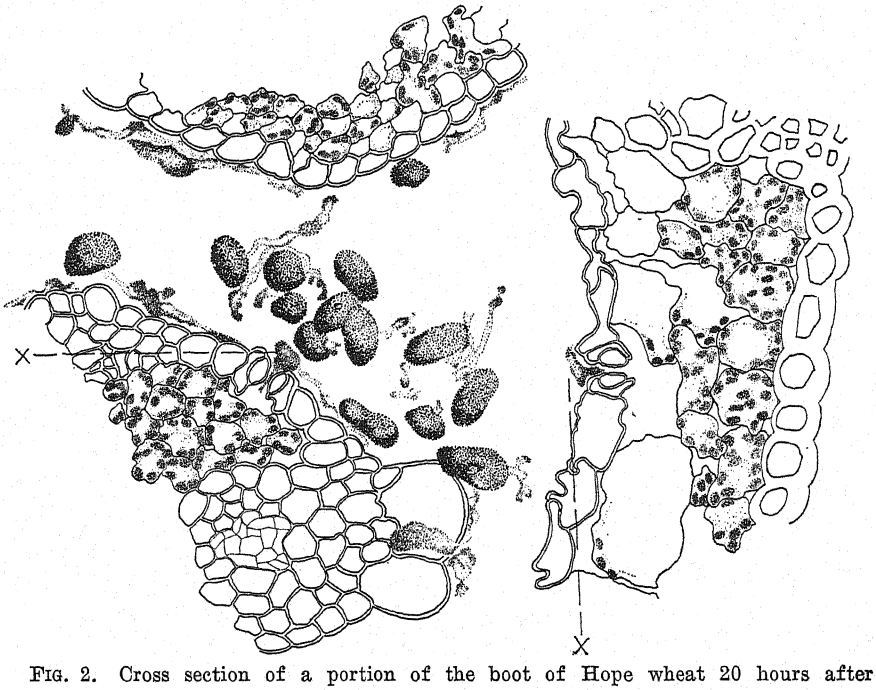


FIG. 2. Cross section of a portion of the boot of Hope wheat 20 hours after inoculation with *Puccinia graminis tritici* 21 by hypodermic syringe. Most of the spores have germinated, and some of the germ tubes have formed appressoria (x) to enter the stomata of the young tissues.

mic syringe was studied histologically and the progress of the rust followed. Spore germination was not inhibited: twenty hours after injection of the spore suspension into the boot of Hope most of the urediospores had germinated and the germ tubes had grown many millimeters (Fig. 2). Some of the tubes were twisted masses in the spaces between sheath and developing head, others had grown along the surfaces of the plant hairs, the awns, or the inner part of the leaf sheaths. There were numerous appressoria over the stomata of the surfaces within the boot, and there seemed no obstacle to entrance of the fungus. The boot provided an ideal moist chamber for spore germination, survival of germ tubes, and the beginning of mycelial infection in the host. Apparently there was nothing to inhibit germination and growth of the inoculum in the Hope host.

A number of investigators (34, 38, 39) found that intense sunlight inhibited spore germination, but it is not likely that this accounts for the lack of infection in older plants of Hope, because seedlings subjected to the same inoculum and the same moist-chamber conditions always were severely infected. To determine whether the inoculum was effective, the epidermis was stripped from the leaf blades of older plants of Hope at various intervals after inoculation. In all cases a relatively large percentage of spores had germinated and formed appressoria over the stomata, so it cannot be said that germination was inhibited.

EFFECT OF INOCULATING OLDER PLANTS BY MEANS OF HYPODERMIC SYRINGE

A number of inoculations by means of hypodermic syringe were made in the greenhouse and in the field to test further the acquisition of rust resistance or the existence of a sharp turning-point with respect to rust reaction as the wheat tissues matured. When the hypodermic syringe was used to force a suspension of urediospores into the rolled leaf sheaths or the boots of Hope plants growing in the greenhouse, the resulting prevalence of infection was usually moderate or severe, and appeared to depend somewhat on the age and succulence of the tissues subjected to attack. The heads inoculated when newly formed and several days before emergence from the boot were completely susceptible and rusted severely in all chlorophyllous tissues. If inoculated just a day before emergence from the protecting leaf sheaths, the infection was light to moderate; some of the uredia were normal in appearance but small, and there were numerous chlorotic areas and evidences of mycelial infections without formation of uredia. Infection of the leaf sheaths, too, seemed severe if the inoculation was made early enough to permit the rust inoculum to reach the young tissues several days before they unrolled. When infection occurred on the expanded leaf sheaths, particularly on the outer layers, it varied from light to moderate, with considerable anthocyanin pigmentation and a browning or blackening of tissues around

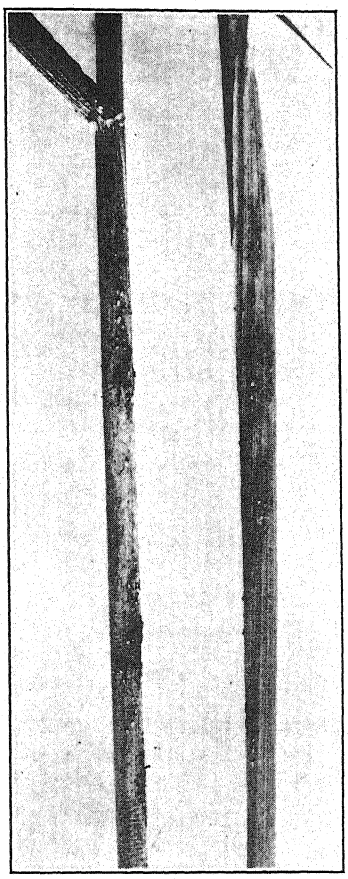


FIG. 3. Maturing plants of Hope wheat rusted by *Puccinia graminis tritici* 21 after inoculation with the hypodermic syringe. Pustules are small, sporulation is scanty, and there is considerable pigmentation about the infection centers when the plants are exposed to intense sunlight.

the pustules (Fig. 3). Dark streaks sometimes appeared on the inoculated leaf sheaths, and one was uncertain, before microscopic examination, whether they were rust infections. Most of them were mycelial infections or subepidermal uredia containing a few spores and a considerable amount of mycelium (Fig. 4, A). A number of the host cells had been killed by the parasite, and the tissues of the infected areas often were filled with materials resembling phenolic deposits. These infections usually remained subepidermal, but sometimes the epidermis was wholly or partly broken and spore production increased slightly. The outward appearance of a pustule was more or less normal, but spore production still was very limited and the

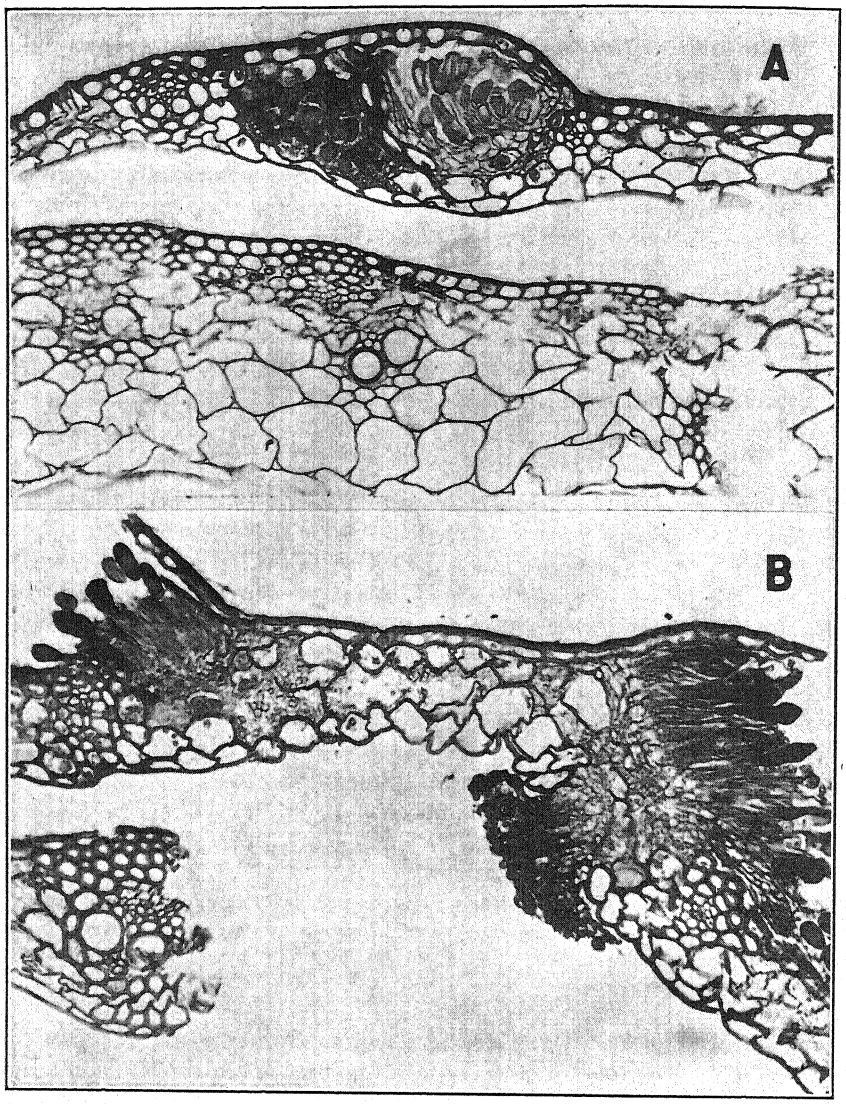


FIG. 4. A. Cross section of leaf sheath of Hope wheat rusted by *Puccinia graminis tritici* 21 after inoculation by hypodermic syringe and exposure to intense sunlight. Subepidermal pustules with abundant mycelium but relatively few spores. Many of the host cells have been killed, and pigments have been deposited in part of the infected tissue. B. Inner rolls of culm of Hope wheat rusted by *P. graminis tritici* 21 2 weeks after introduction of inoculum by hypodermic syringe. Pustules are normal, with abundant mycelium and sporulation, and with very little pigmentation and no destruction of host cells.

surrounding host tissues were brown, black, or deep purple. Only a few pustules ever produced spores in abundance.

Apparently, Hope was definitely antagonistic to rust and repressed its development. It seemed as though Hope acquired resistance to stem rust as its tissues aged, the youngest and most succulent parts of all older plants remaining completely susceptible to rust, while tissues just a few days older resisted infection.

Similar results were secured as a result of hypodermic-syringe inoculations in the field in 1932, but the variation in infection was more pronounced. Occasionally necrotic flecks were the only signs of infection. Eleven days after inoculation, when many uredia normally would be rupturing the epidermis, there often were only chlorotic areas and hypersensitive flecks on the awns and glumes of heads that had emerged from the inoculated boots and a few dark spots on the inoculated leaf sheaths. Infection sometimes occurred on the culm, but the uredia always were extremely small and the surrounding tissues deeply pigmented. Normal, well-developed uredia, however, did not appear on the outer rolls of the culms inoculated in the field during 1932.

Nevertheless, when the inner rolls of the culm were examined a number of normal rust pustules were present (Fig. 4, B), hidden beneath and protected by the outer rolls of the sheath. In such sections there were abundant mycelium and numerous spores, the host cells were not killed, and there was less pigmentation. Host tissue of the same age as that of the outer sheath rolls, in fact the enrolled part of the outer sheath protected by its overlapping margin, permitted normal development of the rust fungus, while the exposed part of the same sheath resisted attack. In the protected inner rolls development was not repressed in any way, possibly because some pathogen had access to the outer rolls and inhibited rust there but was unable to attain the inner rolls, or because some environmental factor affected one tissue more than the other.

EFFECT OF OTHER ORGANISMS ON DEVELOPMENT OF STEM-RUST INFECTION IN HOPE WHEAT

Some of the discolored tissues resembled lesions caused by *Bacterium transluscens* var. *undulosum,* and a few of the blackened pustules looked as though they were parasitized by the bacterium noted by Levine, Granovsky, and Leach (22). Isolations were made from the affected parts to determine whether some other organism was responsible for repression of the rust. In a very small number of the suspected cases the bacterial parasite of rust was isolated, but it was not sufficiently wide-spread to account for the type of rust infection that occurred on Hope wheat. Blackchaff bacteria were never isolated from the rusted culms. Nevertheless, the effect of blackchaff on rust

development was determined by introducing simultaneously a suspension of bacteria from a pure culture of *Bacterium transluscens* var. *undulosum* and a suspension of rust urediospores into young and succulent shoots of Hope wheat plants varying in age from 4 to 10 weeks. Blackchaff symptoms appeared in three days and rust pustules formed in immediately adjacent tissues in six days. Apparently, neither pathogen deterred the other from parasitizing wheat tissue.

A Helminthosporium was isolated from some of the discolored tissues, but it appeared to be a secondary invader. A suspension of its spores, together with a suspension of rust urediospores, was forced into growing wheat plants. Helminthosporium lesions developed not far from the needle punctures, but, as in the case of blackchaff, the development of rust was not inhibited. Apparently, the seeming resistance to rust in older plants of Hope was not due to the antagonism of any other parasitic organism.

Attention then turned to environment and its possible effect on Hope and rust infection. It seemed certain that environment favored spore germination and entry of the parasite, but there was the possibility that establishment and development of the parasite was affected by environment, or that the host was more easily affected by environment and in turn repressed the rust. The outer rolls of the inoculated culms seemed particularly sensitive to light and temperature, for, as was mentioned previously, the leaf blade and part of the sheath often were dry and scalded after a few hot days in the field or after having been in a glass moist chamber in the greenhouse during hot bright days.

It seemed highly probable that some such environmental factor as intense light or high temperature, might decrease the succulence of the exposed plant parts more rapidly and disastrously than it affected the protected tissues, or that the succulence of Hope tissues was more easily affected by environmental factors than were tissues of the other wheats. The fungus could not be expected to develop normally without available water and nutrients. But, when corresponding sections of Marquis and Hope wheats, growing in the field under different conditions of light and soil moisture, were compared as to their free water content, the differences between the two varieties were too slight to account for the repression of rust in Hope and the normal and luxuriant development in Marquis. Hope, when grown at University Farm and not attacked by rust, was not markedly more sensitive to its environment than was Marquis.

There also was the possibility that Hope wheat carried a factor for pigment production under certain environmental conditions and that the pigments in turn prevented the normal establishment of the rust parasite. Broadfoot and Robertson (5) noted the production of pigment in Reward and Marquis wheats, some strains of which seem to carry factors for pigment

production under intense light. In the present work, however, the authors did not observe any pigmentation in nonrusted plants of Hope grown under intense sunlight. Only the plants that had been inoculated with rust produced pigments in the tissues surrounding the center of attack.

Environmental factors influence the parasite directly, but from the observations at hand, it is unlikely that there was an inhibition of spore germination that might explain the lack of infection in so many experiments. Light may repress the mycelial development or may stimulate sporulation before the fungus has had opportunity for ample vegetative growth. But, if such were the case, the phenomenon probably would occur with certain rust forms regardless of the host they were attacking.

Another possibility is that environment affects the combination of host and fungus more easily than it affects either one alone. As soon as parasitic relations are established the two component parts are merged into an entirely new entity. The interplay of environment and the new entity may be quite different from the interplay of environment and fungus or environment and wheat plant. In the present work the two environmental factors, light intensity and temperature, were studied in relation to the development of rust infection in Hope.

DEVELOPMENT OF RUST INFECTION UNDER REDUCED LIGHT INTENSITY

The first experiments under reduced light intensity were made during the last days of March with Hope wheat plants thirteen weeks old, with numerous tillers and with the last leaves shooting, but with no indication of the swollen boot. The day chosen for inoculation was one of alternating cloudiness and diffuse sunlight. The moist chambers were of glass, but they were shaded by screens of a single layer of cheesecloth and not exposed to direct sunlight. The inoculum was rubbed over all plant surfaces with moistened fingers, and care was taken to keep the surfaces moist and the atmosphere saturated, and to keep the temperature as uniform as possible. After 48 hours in the moist chamber the plants were removed but remained screened from direct light. Fortunately, perhaps, there were 16 successive cloudy days following inoculation. After that time the rust infection was well distributed over the entire plant; uredia were scattered from tip to base of leaf blades and over all parts of both lower and upper internodes. Uredia on the more shaded lower internodes were of type 4, while those on the upper parts were generally of type 3 with some chlorosis about each pustule, and occasional uredia were smaller than type 3. The Hope and Marquis seedlings inoculated as checks were severely rusted, with type 4+ and 4++ infection respectively. By the twenty-fifth day all pustules on the older Hope plants were of type 3+ or 4. The period from the twenty-second to the thirtieth day after inoculation was one of bright sunshine and during

that time spore production in the well-established rust pustules was tremendously increased, so that infection was as severe as that that usually developed on protected young and succulent tissues inoculated by hypodermic syringe and approached the severity of infection generally obtained in seedlings.

Later, similar experiments were made, with the light intensity reduced and diffused by cheesecloth screens. Plants in the pre-boot, in the boot, and post-head stages were inoculated, sometimes by brushing with rusted seedlings but more often by rubbing on the inoculum with moistened fingers.

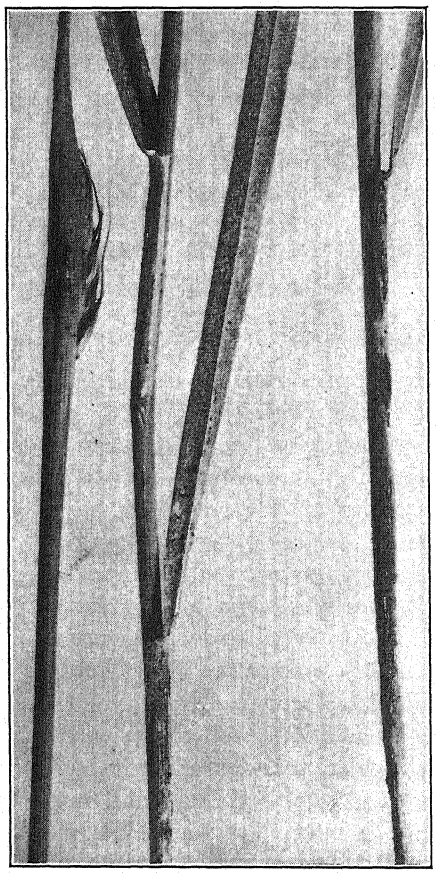


FIG. 5. Culms of Hope wheat rusted by *Puccinia graminis tritici* 21 after inoculum had been rubbed on the plants and the plants shaded from intense sunlight. The two culms at the left were photographed two weeks after inoculation before the fungus reached the height of its spore production. The culm at the right was photographed 4 weeks after inoculation, and, although the ruptured pustules were not large, spore production was so great that the entire culm was covered with the spore mass.

Check plants, either seedlings or plants 3 to 4 weeks old, always were inoculated and incubated with the older plants. In some experiments spore germination was tested as well. Inoculations were made on cloudy days whenever possible, and in a number of cases the stomata were examined during the first few hours of the moist-chamber period. Rust pustules first appeared 8 to 9 days after inoculation on the shaded plants, but the infection did not attain its greatest severity until 3 to 4 weeks after inoculation (Fig. 5). All plant parts that were green and succulent at the time of inoculation proved to be susceptible to rust attack. The largest pustules were on the more shaded parts and those that seemed to retain their succulence longest: the extreme base of the leaf blade, at the juncture of the blade and leaf sheath, and the leaf-sheath tissue just above the nodal swellings throughout the lower half of the culm. The effect of light intensity was particularly striking when infection on shaded plants was contrasted with the infection on similarly inoculated plants exposed to full and direct sunlight a few days after removal from the moist chamber. The necrosis and the hypersensitive areas and the definite pigmentation on the latter were noticeable about 10 days after inoculation. On the shaded plants rust infection developed slowly but certainly, and after 3 to 4 weeks urediospore production was so great that the entire culm seemed covered with the spore mass.

When rust inoculum was forced into the expanding shoots by means of a hypodermic syringe, all parts of the shaded older plants were completely susceptible to rust, and severe infection developed within two weeks after inoculation (Fig. 6). Type 4 pustules predominated, and there was no indication of resistance to rust. The outer culm tissues, as well as the protected inner sheath rolls, were severely rusted, and there was no evidence of pigmentation or hypersensitiveness. Hope seems to have no protoplasmic resistance to *Puccinia graminis tritici* 21 when light intensity is reduced.

There are indications that other fungi may have optimum light intensities for development on their respective hosts. Volk (35) found that a slight reduction in light intensity allowed a more luxuriant growth of *Erysiphe graminis* on rye but hindered the infection of rye by *Puccinia dispersa.* He also obtained better development of the facultative parasite *Cladosporium fulvum* on tomatoes when the light intensity was reduced slightly.

The development of rust infection usually is slower in older plants than in seedlings, partly because of the differences in cell-wall structure of the rust-susceptible parts, partly because of the differences in the plant colloids and their effect on the water relations and nutrition of the parasite. It is possible that environmental factors, such as light intensity, may affect the normal rate of change in host constitution sufficiently to permit luxuriant



FIG. 6. Hope wheat plants rusted by *Puccinia graminis tritici* 21 after inoculation by hypodermic syringe. Plants were shaded from intense sunlight after inoculation and all parts proved to be completely susceptible to rust. Most of the pustules were large, well-developed, and sporulated abundantly.

development of the pathogen at one time or to impede its development at another. Temperature, likewise, may affect the compatibility of parasite and host, particularly in certain delicately balanced parasitisms.

EFFECT OF HIGH TEMPERATURE ON RUST INFECTION

Older plants of Hope were inoculated with *Puccinia graminis tritici* 21 by rubbing urediospores over the moistened plant surfaces. Plants were in the pre-boot, the early boot, very late boot, or had headed just a day or two before inoculation. Inoculated plants were placed in moist chambers, shaded from direct light, and kept at 24° C., to permit spore germination and entrance of germ tubes. After 48 hours the plants were placed on the greenhouse bench and again shaded. The crocks were buried in moist sand so that the soil might be slightly cooler than the atmosphere, but the daily air temperatures in the greenhouse at this time usually varied from 26° to

33° C., and the relative humidity of the air seldom was more than 20 per cent. Light rust infection appeared thirteen days after inoculation. At that time there were chlorotic and necrotic areas on the awns, glumes, and peduncles that had been inoculated and on a few of the leaf blades and sheaths. Small pustules appeared scattered over the lower internodes, on the upper internodes just above the nodal swellings, on some of the leaf blades, and on one glume. Fifteen days after inoculation it was evident that the plant tissues were drying as a result of the high temperature, despite the shade and an abundant supply of soil moisture. Rust infection was light, and the severity did not increase during four weeks after inoculation. At that time many of the host tissues were thoroughly dry and scalded and rust was evident only on those parts that had retained their chlorophyll and some degree of succulence. There were a few groups of pustules of type 3 = on the north side of two of the greenest culms, but most of the pustules were minute and there was an appreciable amount of pigmentation and chlorosis. A number of infected areas were blackened but spores never appeared, and hypersensitive flecks were common. The infection resembled that under intense light.

This is further evidence that environmental factors influence the complex and dynamic phenomenon of parasitism and that each combination of host and parasite must be considered individually, as an optimum environment for one combination may be detrimental to another. In this respect Hope wheat and *Puccinia graminis tritici* 21 seem to be more easily affected by environment than some of the other combinations studied. Furthermore, Smith (29) has shown that, in Washington, the combination of Hope wheat and *Tilletia tritici* is greatly affected by environment, especially by temperature. Spring-sown Hope is resistant to bunt; when fall-sown it is susceptible. Smith found that temperature is likely to rise very soon after emergence of wheat seedlings in the spring, but in the fall the rise in temperature is delayed for several months. In his experiments high temperatures checked the development of bunt in Hope wheat but not in the variety Jenkins. Smith thinks that the internal composition or the protoplasmic organization of Hope at low temperatures permit the fungus to develop, but that the different composition at high temperatures inhibits or retards the fungus.

DISCUSSION.

The foregoing observations support and strengthen the opinion that a host-parasite complex is affected quite as much by environmental factors as are its component parts. It is probable that the balance between host and parasite may be affected more easily than has been realized in the past. The micro-environment, particularly, may determine the develop-

ment of an individual rust infection and the host's reaction to its parasite. In the past it often has been the practice to study the direct effect of environmental factors on the parasite or on some phase of the infection, and to consider the host in its protoplasmic constitution, its vigor, or its physiological development, merely as another factor influencing the progress of the parasite. Recently, however, Johnson (19) has called attention again to the necessity of considering the host-parasite complex when studying cereal rusts, recognizing the fact that environmental factors may affect the interactions of host and fungus.

The combination of Hope wheat and *P. graminis tritici* 21, as studied in the present work, seems to be easily affected by its environment. There was the possibility that the metabolism of Hope, a variety produced by crossing an emmer and a common wheat, was more affected by environment than were metabolic processes of other varieties, and that the resulting changes in the host in turn affected the development of the fungus. This view is supported in part by the work of Smith (29), but in the present work, Hope, without its stem-rust parasite, is less sensitive to its environment than is the host-parasite complex.

Non-rusted Hope plants under intense sunlight did not form the pigments that so generally characterized the tissues surrounding rust infections in Hope. The presence of the parasite was essential for the coloration, intense light alone being insufficient stimulus for the reaction. Nor was the parasitic presence alone responsible for the coloration, because, with reduced light intensity, the pigments were not produced.

Hope seems less resistant to drought than do some of the other wheats; and it was thought at first that the rapid drying and decrease in succulence of the leaf sheaths might be peculiar to Hope, even when there was no opportunity for rust infection. This was not true, however, for when free moisture contents of various tissues were compared in the varieties Marquis and Hope, the determinations ran approximately parallel throughout the important part of the growing season, from the time the plants were in the jointing stages until after flowering. Here, too, both the presence of the fungus and the intense sunlight were required to bring about the rapid drying of tissues.

The interactions of rust and wheat cells are not at all clear and at present it is impossible to explain the effect of external factors on the complex. It may be that the attack of the parasite is so violent and rapid as to destroy the photosynthetic apparatus of the host cells and make it impossible for them to assimilate materials for their own tissues and the fungus as well. It may be that the rust upsets the water relations or nutrient conditions of the wheat tissues. If the fungus appropriates water or nutrients faster than the supply required by the host can be replenished.

it would not be surprising if the host tissues dried out before the fungus was able to complete its development and sporulate. There is the possibility also that the establishment of the parasite or any disturbance by it of water or nutrient relations of the host plant might also affect the rate at which the colloidal materials of the wheat change; and, after the initial disturbance, external environmental factors in turn may have a greater effect on the colloidal condition and processes of change in the protoplasm. Undoubtedly, changes in metabolic rates or disturbances of the physiological processes in host and parasite influence the compatibility of the two organisms. In the light of recent work it is impossible to say whether the fungus entity or the host entity is the more important. Both are requisite and, in the opinion of the writers, probably should be considered as a complex in the future studies on the nature of rust resistance.

Other workers also have noted changes in resistance to rusts as the environmental factors of light and temperature varied.

From epidemiology studies of *Puccinia graminis* and physiologic-form surveys made annually in the Mississippi Valley, it is evident that environmental conditions affect rust infection, sometimes to such an extent that it is difficult to distinguish between certain physiologic forms. Stakman, Levine, and Wallace (32) point out that forms 11 and 32 of *P. graminis tritici* might easily be confused under certain environmental conditions. Form 11 produces a type 4 infection on the durum differentials, which may appear like the type X produced by form 32 unless environment is very favorable for rust development. The same difficulty is encounterd in differentiating forms 38 and 39 (see Stakman and Levine, 31), for the type X infection produced on the durum differentials by form 38, under certain environmental conditions, is not easily distinguished from the type 4 infection produced by form 39. Wallace (36) also has discussed the similarity of forms 18 and 36, which are identical in their behavior on all differential varieties with the exception of Kubanka. Form 18 produces type 4 infection on Kubanka and form 36 the type X infection, but, under certain environmental conditions, the flecks and small pustules of the type X infection seem to be masked by the large pustules, and form 36 produces what appears to be type 4 infection on Kubanka. Wallace had difficulty distinguishing between forms 18 and 36 in the physiologic-form surveys of 1926 and 1928.

Johnson (19) also believes that many of the wheat varieties now commonly grown may vary in their rust resistance in accordance with weather conditions. He decided that temperature, and probably light, influenced profoundly the X reaction produced on both seedlings and older plants of durum wheat varieties with a number of physiologic forms of *P. graminis tritici*. Under low light intensities the typical X reaction was produced at the intermediate temperature of 65° F., but at lower temperatures (55 to

60° F.) the reaction was commonly 0; or X, while at higher temperatures (70 to 75° F.) the reaction usually was 4. An increase in light intensity permitted the development of the typical X reaction at the lower temperatures, while the increase in temperature brought about the development of the 4 reaction. Johnson's results agree with those of Waterhouse (37) in Australia, who found that seedling reactions with certain physiologic forms of *P. graminis tritici* varied from type 0; in winter to a type X in spring and to a type 4 in summer.

Gordon (12) reports that Joanette strain of oats varies greatly in its reaction to certain forms of *Puccinia graminis avenae,* being extremely resistant to form 4 at 57.4° F., and completely susceptible to the same form at 75.4° F.; and Peturson (26) obtained similar results with some physiologic forms of *P. coronata avenae,* three oat varieties being completely susceptible to form 7 at 77° F., very resistant to the same form at 70° F., and practically immune from this form at 57° F.

The work done by Melander (23) with *P. graminis tritici* indicated that at the very low temperature of 0 to 1° C., physiologic form 35 on Little Club wheat and form 15 on some of the wheat differentials produced pustules approaching a type 1 infection. This type prevailed as long as the wheat seedlings were kept at the low temperature, but if they were transferred to 20° C. the pustules changed to type 3 or type 4 uredia.

Thus in some rusts relatively high temperatures are necessary for susceptibility with the complete development of the parasite, but low temperatures tend to swing the reactions towards resistance. On the other hand, high temperatures may be detrimental to the development of rust infection and thus be responsible for a field resistance to rust. In Germany Gassner and Straib (10) found that development of and the degree of resistance to *P. glumarum tritici* depended greatly on temperature relations: wheat varieties resistant at 20° C. were severely rusted at slightly lower temperatures. Radelescu (27) believes that external factors may affect and change the physiologic resistance of some wheat varieties to *P. glumarum tritici,* and his work indicates that temperature has been one of the primary factors responsible for the field resistance of wheat to stripe rust in Germany. In the western provinces of Canada, too, temperature seems to have a profound effect on the reaction of wheat varieties to stripe rust. Newton, Johnson, and Brown (25) tested a number of varieties and found them all resistant to stripe rust at 78° F., but that many varieties were completely susceptible at 54° F.

Similar observations have been made for leaf rust of wheat. Gassner and Straib (11) found that certain wheat varieties that were highly resistant to *P. triticina* 14 at about 20° C. were completely susceptible to form 14 when cultured at lower temperatures.

In many of the cases cited it is impossible to decide whether the effect of temperature is on the parasite or on the host-parasite complex. The effect of temperature on germination of spores and growth of germ tubes of various rusts is definite and well established; but as soon as the parasitic relationships are set up in the host plant it is impossible to consider the rust as a separate entity any longer.

Miss Forward (7) approached the problem of rust resistance from the standpoint of the host plant rather than the pathogen, attempting to study the physiological basis of compatibility and the modifiability of infection type by altering the metabolism of the host. She has reported upon the modification of infection by *P. graminis tritici* when the host metabolism is altered by extended periods of darkness or darkness broken by short exposures to light. There is a loss of compatibility between fungus and host, and resistant symptoms appear, with an interposition of a more or less extended period of darkness at the beginning of pustule formation. On the other hand, darkness broken by a daily 4-hour exposure to light did not visibly alter the metabolism of the host, and rust developed as rapidly under such conditions as when plants were exposed to light all day.

Puccinia glumarum, too, in its uredial stage is affected by light as well as by temperature, for Bever (4) found that with a day length of 15 hours or more the infection type on Pannier barley changed distinctly from type 4 to type 0. Evidently there is an optimum light intensity for development of stripe rust in the field, for Bever mentions that a day length of less than 12 hours lengthened the incubation period for rust in his experiments; and he believes that the tardy spring advent of stripe rust that sometimes occurs in Idaho during cloudy and rainy seasons is due to the reduction in light intensity below the optimum rather than to variations in temperature.

The present work also supports the view that parasitic relations may be profoundly affected by environment; and it emphasizes the fact that the influence exerted by a particular factor of the environment varies greatly according to the rust species considered, and even according to the different combinations of certain physiologic forms of a single rust species and their host varieties. It seems that every individual host-parasite complex has its optima with respect to various environmental factors—optima that may be nearly identical with the optima for numerous other host-parasite complexes, or may be at variance with those for most combinations.

Much investigational work remains to be done in determining the nature of rust resistance. So far, it has been necessary to observe the phenomenon of parasitism as it occurs and varies in nature or as it has been modified by experimental procedure. The ultimate basis for the compatibility or incompatibility of obligate parasites and their hosts is still to be demonstrated.

SUMMARY

The present work offers evidence that there is an optimum light intensity for mycelial development and sporulation of *Puccinia graminis tritici* in Hope wheat.

Under normal intense sunlight of the Upper Mississippi Valley plants of Hope wheat in various stages of development beyond the seedling stage appear to have considerable resistance to *Puccinia graminis tritici* 21.

When light intensity is reduced by shading the plants they are completely susceptible to *P. graminis tritici* 21, just as they are completely susceptible in their seedling stages.

High temperatures have an effect similar to that of intense sunlight, so that plants appear resistant to rust.

The external environmental factors, light and temperature, seem to influence the host-parasite complex rather than its component parts.

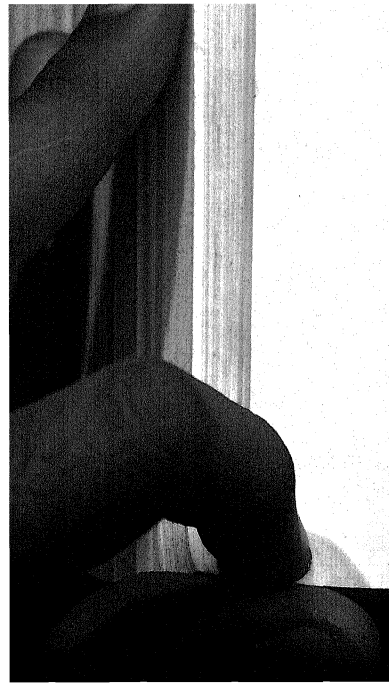
The authors are very grateful to Dr. E. C. Stakman for his counsel and critical advice throughout the investigations and to Dr. J. J. Christensen for his helpful criticism of the manuscript.

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STUDIES ON FRENCHING OF TOBACCO[1]

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Among the so-called physiological diseases of tobacco, frenching is one of the most puzzling so far encountered by plant physiologists and pathologists. In Princeton this disease proved to be severe in the winter of 1932 in tobacco grown in greenhouses. During the following summer it occurred extensively in plants in an experimental field. Since that time numerous greenhouse experiments have been carried out in an endeavor, firstly, to prevent the disease, and secondly, to determine factors favoring its development. The object of this paper is to present the results of the experiments and such conclusions as could be drawn from the experimental data.

LITERATURE

Frenching of tobacco is very widespread in occurrence, as its presence has been observed in most of the large tobacco-growing areas of the world. In the United States frenching has been reported in Connecticut (4), Florida,[2] Georgia,[2] Indiana,[3] Kentucky[2] (5), Louisiana (13), Maryland[2] (13), Massachusetts,[2] New York,[4] North Carolina[2] (13), Ohio (13, 18), South Carolina,[2,5] Virginia[2] (13), West Virginia,[6] and Wisconsin.[2] It has been reported in each of the following foreign countries: Canada,[7] France[2] (17), Italy (22), Russia[2] (17), Dalmatia[2] (17), Rhodesia (9), Transvaal (1), West Africa,[2] and Java[2] (17).

The earliest reference to frenching found in the literature was in a letter by John Clayton (6) to the Royal Society, May 12, 1688. Symptoms of frenching have been described by a number of early workers, including Dodge (5), Killebrew (13), and Allard (2). Clinton (4), in 1914, demon-

[1] Published at the expense of The Rockefeller Institute for Medical Research, Princeton, N. J., out of the order determined by the date of receipt of the manuscript.

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strated that frenching cannot be transmitted to healthy plants by inoculation, thereby showing that it is a noninfectious disease and not a severe form of mosaic. His findings were corroborated by Wolf and Moss (26) in 1919, and by Garner (7) in 1922. Johnson (11) observed that poor soil aeration and low soil fertility were contributing factors favoring the development of the disease.

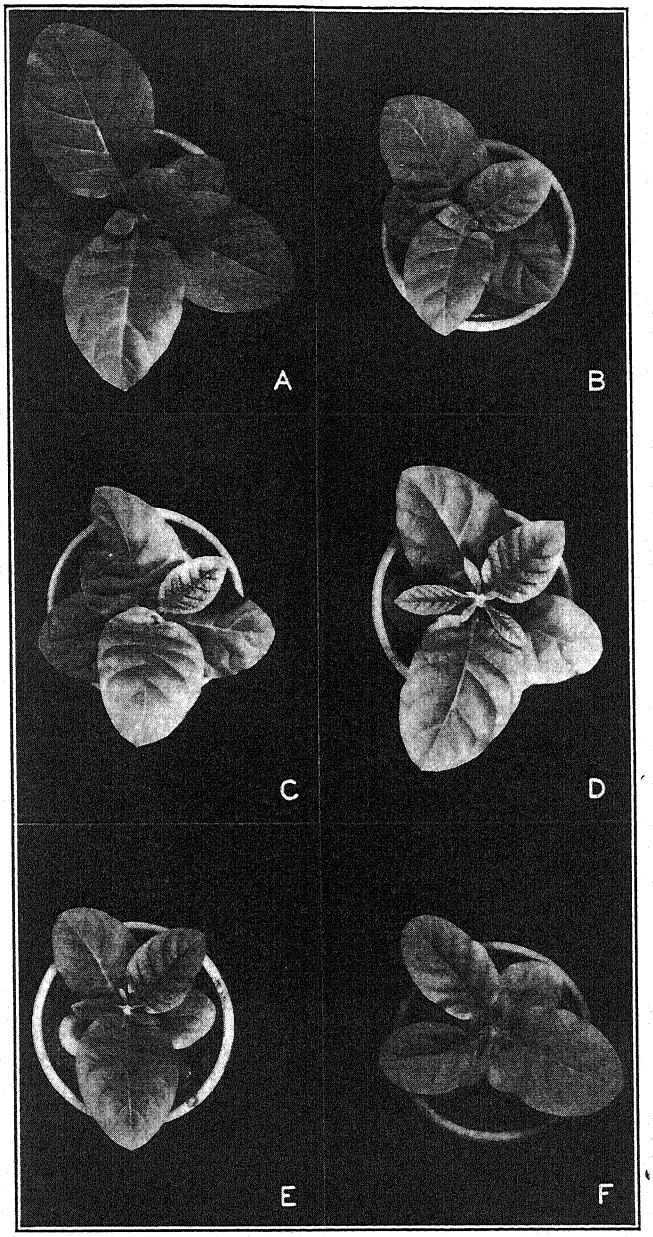
The suggestion that frenching is a nitrogen-deficiency disease was first advanced by Valleau and Johnson (23) in 1926. They concluded that it is the result of an accumulation of carbohydrates brought about by the absence of sufficient available nitrogen. This physiological condition was conceived to give rise to the development of new leaf tissue deficient in chlorophyll. In a later paper they (24) reported that frenching could be readily controlled by the addition of available nitrogen. This conclusion was confirmed by Haas (8) in California and by Hopkins (10) in Southern Rhodesia.

Morgan (16) in 1929 questioned the hypothesis that frenching is a nitrogen-deficiency disease. He was able to show that it developed when various fertilizer mixtures were added to the soil and was not restricted to mixtures deficient in nitrogen. In a later publication Valleau and Johnson (25) modified their original conception that frenching is a nitrogen-deficiency disease by suggesting that it is not caused directly by a deficiency of nitrogen alone, but probably by a lack of balance between translocation of carbohydrates and of nitrogenous compounds within the plant. This condition may be brought about by a deficiency of any nutrient element essential for protein metabolism. In a more recent report Karraker and Bortner (12) suggested that, since frenching is obviously a disease of the growing points of the plant, its expression is the result of a retardation in the translocation of food materials to and from these growing regions.

The possibility that frenching is a toxicity disease rather than a deficiency disease was first expressed by McMurtrey (15) in 1932. He reported in a brief note that symptoms of thallium toxicity in solution culture resembled those characteristic of frenching, although not identical in all respects. Shear (19) also suggested that frenching might be a toxicity disease, but advanced no theory as to the nature of the toxic factor. No conclusive experimental evidence has been presented heretofore to show that frenching is a toxicity disease.

SYMPTOMS

Since frenching has been adequately described by Clinton (4), Shear (19), and Valleau and Johnson (24, 25), only a brief description of the most characteristic symptoms will be given here. The earliest symptom to be observed is chlorosis along the margins of young leaves (Fig. 1, B). This gradually spreads toward the midribs and, finally, the entire interveinal



Photographed by J. A. Carlile

FIG. 1. Tobacco plants showing characteristic symptoms of frenching. A, healthy check plant; B, first symptoms with interveinal chlorosis along margin of leaf; C, interveinal chlorosis extending over entire leaf; D, elongating leaves, forming the strap-leaf stage; E, severe strap-leaf condition; F, stimulation of axillary buds forming a rosette of many, small, strap-shape leaves.

regions are involved (Fig. 1, C). The network of veins, however, remains dark green. As the leaf develops, only the midrib elongates apparently, thereby producing a long narrow, ribbon-like or strap-shape structure (Fig. 1, D). Terminal growth is greatly retarded, resulting in a stunted plant with small, brittle, distorted leaves (Fig. 1, E). In severe cases the axillary buds are stimulated into growth, producing a rosette of small, narrow, strap-shape leaves (Fig. 1, F). These narrow leaves are very light in color. Frenching is not confined to the seedling stage of growth, but may appear at any stage up to flowering. It is sometimes accompanied by a yellowing of the lower leaves.

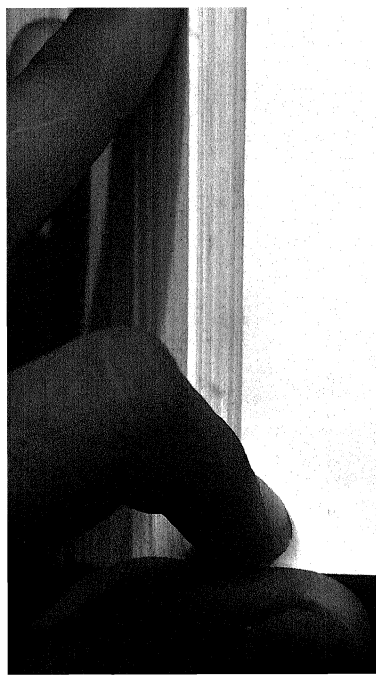
Symptoms of the disease are noticeable also in the roots. The diameter of the old roots is reduced to about one-half that of normal roots of the same age. The number of root hairs is markedly decreased; in some cases they are completely lacking. The cortex near the root tips is pronouncedly discolored and necrotic. Root systems of frenched plants usually are more profusely branched than those of healthy plants.

EXPERIMENTAL

Numerous greenhouse experiments were carried out in an endeavor not only to control frenching of tobacco but also to determine its causal factor. Since frenching developed very severely in a near-by field, soil from this area was used in all of the experiments except where mention is made of soil from another source. This soil, which will be referred to as field soil, was quite heavy in texture, of high water-holding capacity, and of low fertility, with a neutral or slightly alkaline reaction.

An experiment was first carried out to determine what plants were susceptible to frenching. All seedlings were germinated in sand and then potted in field soil in 4-inch pots. In the first test, seedlings of 16 varieties of *Nicotiana tabacum* L. were used. These varieties, all of which developed frenching within 6 weeks, were Adcock, *angustifolia, auriculata,* Burley, *calycina, chinensis, colossea,* Connecticut Seed Leaf, *gigantea, latissima,* Little Orinoco, *macrophylla,* Maryland, *purpurea,* Turkish, and Zimmers. In the second series 18 different species of the genus Nicotiana were tested. The species that developed frenching were *N. alata* Link and Otto, *N. langsdorffii* Schrank, *N. longiflora* Cav., *N. rustica* L., *N. sanderae* Sander, and *N. sylvestris* Spegaz. and Comes. The following species showed no symptoms of frenching: *N. acuminata* Hook., *N. bigelovii* S. Wats., *N. caudigera* Phil., *N. clevelandii* A. Gray, *N. glauca* R. Grah., *N. glutinosa* L., *N. palmeri* A. Gray, *N. paniculata* L., *N. suaveolens* Lehm., *N. tomentosa* Ruiz and Pav., *N. trigonophylla* Dun., and *N. wigandioides* C. Koch and Fint.

Petunia hybrida Vilm. var. Henderson's Giant Empress, *Datura stramonium* L. (Jimson weed), and *Lycopersicon esculentum* Mill. var. Bonny Best



(tomato) were the only other plants in the Solanaceae that showed characteristic symptoms of frenching. The chlorosis in these three species, however, was mild. The solanaceous plants that gave negative results were *Capsicum frutescens* L. (pepper), *Hyoscyamus niger* L. (henbane), *Lycium ferocissimum* Miers., *Nicandra physalodes* (L.) Pers., *Physalis angulata* L. (ground cherry), *Solanum melongena* L. (eggplant), and *Solanum tuberosum* L. (potato).

None of the non-solanaceous plants grown showed any symptom of frenching. The species tested were *Zea mays* L. var. *rugosa* (field corn), *Fagopyrum esculentum* Gaertn. (buckwheat), *Beta vulgaris* L. (beet), *Spinacia oleracea* L. (spinach), *Vicia sativa* L. (vetch), *Glycine max* Merr. (soybean), *Medicago sativa* L. (alfalfa), *Phaseolus vulgaris* L. var. Early Golden Cluster (bean), *Tropaeolum majus* L. (nasturtium), *Gossypium hirsutum* L. (cotton), *Begonia semperflorens* Link and Otto (begonia), *Petroselinum hortense* Hoffm. var. *crispum* (parsley), *Salvia splendens* Ker. (scarlet sage), *Cucumis sativus* L. (cucumber), *Callistephus chinensis* Nees (aster), and *Lactuca sativa* L. var. *crispa* (lettuce).

A number of experiments were made in an endeavor to determine whether frenching is a parasitic, deficiency, or toxicity disease, since it seemed probable that it would fall into one of these three categories. Seedlings of *Nicotiana tabacum* var. Turkish were used as test plants in the experiments described below.

Parasitic Studies

Although Clinton (4), Wolf and Moss (26), and Garner (7) reported that frenching is not a parasitic disease, these investigators did not present the experimental evidence upon which their conclusions were based. Several experiments were made in an attempt to verify this conclusion. A microbiological examination of roots of field-grown healthy and diseased tobacco plants failed to show any association whatsoever between the disease and a pathogen. The following greenhouse experiments substantiated this observation.

When frenched plants were repotted in a mixture of 99 per cent sand and 1 per cent soil, they recovered temporarily within a few days, but became frenched again within 4 weeks. However, similarly diseased plants repotted in 100 per cent sand recovered and remained healthy till the experiment was discontinued. If the disease is due to a parasite, it is difficult to understand why those plants repotted in 100 per cent sand recovered permanently, whereas those placed in 99 per cent sand recovered only temporarily. Sufficient organisms should have been transferred on the roots of the frenched plants in the 100 per cent sand to cause a reoccurrence of the disease, if it is due to a parasite.

In another experiment roots from frenched plants were cut into small pieces and mixed thoroughly with washed sand. Healthy seedlings potted in this mixture and watered with tap water showed no evidence of the disease. When frenched plants were repotted in this mixture, they recovered and remained healthy. When a frenched plant was repotted in a pot of washed sand, in which a healthy plant was already growing, the healthy plant not only remained healthy but the repotted, diseased plant recovered and thereafter showed no symptoms of the disease.

Moreover, soil sterilization did not prevent the development of frenching completely, as would be expected if the disease were caused by a parasite. Soil was sterilized with steam at 15 pounds' pressure in an autoclave for 1 hour on 3 consecutive days. After the 3rd period of sterilizing, samples of soil were plated on agar plates. No growth of any sort developed on the plates within 14 days. Plants potted in this sterilized soil showed the first symptoms of frenching from 2 to 4 weeks later than they did in nonsterilized soil, but eventually all the plants became frenched. This delay in the appearance of symptoms may be explained as follows. Plants in sterilized soil grew faster than similar plants grown in nonsterilized soil, indicating that during the process of sterilization certain organic nitrogenous compounds might have broken down and thereby liberated nitrogen, which became available for plant growth. Therefore, the appearance of frenching was delayed until some of this nitrogen had been used up. The addition of small amounts of nitrogen to soil has been shown to retard the development of frenching. From this experiment and the 3 preceding ones, it appears improbable that frenching is a parasitic disease.

Physiological Studies

Studies were undertaken to determine whether frenching is a deficiency disease or a toxicity disease. Before these studies were carried out, however, it seemed advisable to ascertain the importance of such soil factors as texture, aeration, and reaction in making conditions favorable for the development of frenching.

In the first experiment dealing with soil texture, various amounts of peat and sand were mixed with field soil. Young seedlings were then potted in the mixtures in 4-inch porous clay pots and kept well watered throughout the experiment. It was found that the incidence of frenching in various mixtures of sand and soil was equal to, if not slightly higher than, the incidence of disease in field soil alone. Peat and soil mixtures, on the other hand, lowered the incidence of frenching very noticeably. The number of plants that became frenched decreased as the proportion of peat in the mixture increased. When 50 per cent by volume of the mixture was peat, all plants remained healthy. Neither incidence nor severity of frenching could

be correlated definitely with rapidity of growth. With another set of plants an experiment was made to determine the effect of variations in the amount of watering. When the plants were watered heavily, 75 per cent of them became frenched. With light watering, however, plants of a similar age remained healthy.

Since increasing proportions of peat in soil mixtures decreased and heavy watering increased the incidence of frenching, it was of interest to determine if aeration was a factor in the development of frenching. One set of plants was potted in 100 per cent sand, and a similar set in 99 per cent sand and 1 per cent field soil by volume, in 4-inch pots placed in saucers. The saucers were kept partly filled with water at all times. Within 3 weeks after potting, all the plants in 99 per cent sand and 1 per cent soil became frenched, whereas those potted in sand alone remained healthy even when held under observation in a greenhouse for nearly 4 months. For all practical purposes the aeration and texture of the rooting medium in these two treatments were equal, indicating that frenching, under certain conditions, can develop even with good aeration.

The effect of soil reaction in promoting or retarding the development of frenching is brought out in the following experiment. Various amounts of pulverized lime were thoroughly mixed in a soil that does not produce frenching under ordinary conditions. Young seedlings were potted in these soil mixtures in 4-inch pots placed in saucers that were kept filled with water. The number of plants that developed frenching was observed at weekly intervals. None of the plants became diseased until the end of the 6th week. When the experiment was terminated after 11 weeks, the soil reaction was determined and the green weight of all plants measured. The experimental

TABLE 1.—*Effect of the addition of lime on the development of frenching*

Amount of lime added gm./pot	Number of plants	Number of plants frenched after:						Records after 11 weeks	
		6 weeks	7 weeks	8 weeks	9 weeks	10 weeks	11 weeks	Soil reaction pH	Green weight of plants
0.0	5	0	0	0	0	0	0	7.67	5.0
0.5	5	0	0	0	0	0	0	7.80	7.1
1.0	5	1	1	1	3	5	5	7.90	10.5
1.5	5	2	2	4	4	4	4	8.00	10.0
2.0	5	1	2	2	3	3	3	8.13	14.1
3.0	5	0	0	0	1	1	1	8.23	12.3

data in table 1 show that there was a definite correlation between the hydrogen-ion concentration of the soil and the development of frenching. At pH

7.7 and 7.8 none of the plants became frenched, but at pH 7.9 and 8.0 nearly all the plants developed the disease. Above pH 8.0 a few plants became frenched, but the number decreased as the soil reaction became more alkaline. Even at pH 8.2 the plants made good growth, indicating that the absence of the diseased condition at this reaction was not due to poor growth. The range of soil reaction most favorable for the development of frenching was found to vary somewhat, depending upon other environmental conditions.

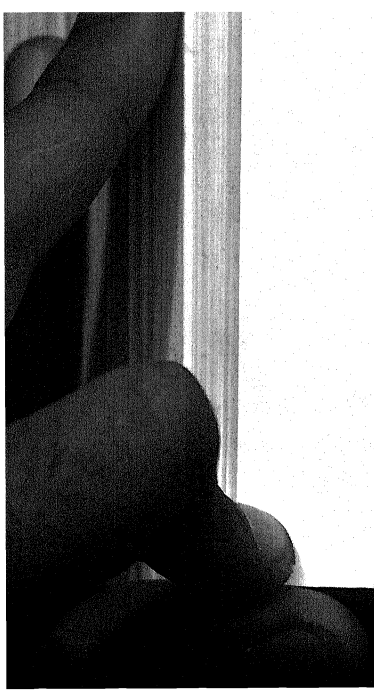
The preceding experiments show that soil texture, aeration, and reaction are important secondary factors in the development of frenching. They exert a pronounced action in modifying the incidence and severity of the disease.

Deficiency Studies. In order to determine if frenching might be a deficiency disease, a study was made of the effect on tobacco in sand cultures of a deficiency of the elements known to be essential to plant growth. The nutrient solution was added continuously by means of the constant-drip method of solution renewal (20). Typical symptoms of nitrogen, phosphorus, potassium, calcium, magnesium, and sulphur deficiencies were obtained, but none of these symptoms resembled those characteristic of frenching.

In order to determine the effect of the deficiency of some of these elements when seedlings were grown in soil, a repetition of the above experiment was made, using field soil instead of sand as the growing medium. The nutrient solution was added twice each week, 100 cc. per pot. The plants became frenched when nitrogen or potassium was omitted from the nutrient solution added. In these particular deficiency treatments the plants grew very slowly. Typical symptoms of nitrogen- or potassium-deficiency, in addition to the characteristic symptoms of frenching, were obtained.

These experiments with sand and soil indicate that frenching does not result from a deficiency of any one of the principal essential elements. Since it occurred in soil with the addition of potassium in a nitrogen-deficient nutrient, and also with the addition of nitrogen in a potassium-deficient nutrient, neither a nitrogen nor a potassium deficiency can be considered as the factor causing the disease.

Experiments were then carried out to determine the effect of the elements known to be required for growth in small quantities, such as iron, boron, and manganese. Plants were grown in liquid nutrient cultures in which one of these elements at a time was omitted. None of the symptoms obtained, however, resembled those of frenching. Symptoms other than those of frenching were obtained when an excess of any one of these 3 elements was added. Various amounts of iron, boron, and manganese when added to soil failed to prevent the development of frenching or to bring about recovery when added to soil in which the plants were already frenched.



Since it has been shown by some workers (3, 14, 21) that copper exerts a stimulatory action on plants under certain conditions, tests were made on the effect of copper sulphate on the development of frenching. Table 2

TABLE 2.—*Effect of repeated additions of copper sulphate on the prevention of frenching*

Treatment	$CuSO_4$ (1%) added each week (cc./pot)	Number of plants	Number of plants frenched after:						
			16 days	17 days	20 days	35 days	50 days	65 days	80 days
A	0	5	3	5	5	5	5	5	5
B	5	5	0	3	5	5	3	1	0
C	10	5	0	1	2	2	1	0	0

gives the results of an experiment in which various amounts of $CuSO_4$ were added to field soil. The first treatment was made 5 days after the plants were potted. In group C the $CuSO_4$ exerted some toxic action as the plants were much smaller than those in group B. However, group A and B plants were about equal in size. In group A the symptoms of frenching were very severe. The plants in group B, however, showed only slight chlorosis after 20 days, but by the end of the experiment they appeared to be healthy again. Table 3 shows the effect of adding $CuSO_4$ to plants after

TABLE 3.—*Effect of repeated additions of copper sulphate on the recovery of plants from frenching*

Treatment	$CuSO_4$ (1%) added each week (cc./pot)	Number of plants	Number of plants frenched after:				
			16 days	35 days	50 days	65 days	80 days
D	0	5	5	5	5	5	5
E	5	5	5	5	4	2	0
F	10	5	5	3	2	0	0

they had become frenched. The plants in groups E and F recovered completely, while those in group D showed the rosette condition severely throughout the experiment. The experiments show quite clearly that $CuSO_4$ had an appreciable effect in overcoming frenching and bringing about recovery from the diseased condition. The $CuSO_4$ exerted no measurable change on the hydrogen-ion concentration of the soil.

In order to determine if aluminum had any effect on the development of frenching, various amounts of aluminum sulphate were added to 4-inch pots of field soil. The results obtained when $Al_2(SO_4)_3$ was added at weekly

Photographed by J. A. Carlile

FIG. 2. Plants showing the effect of aluminum sulphate on prevention of frenching. G, check plant on left showing severe frenching; H, plant that received 1 gram $Al_2(SO_4)_3$ showing mild chlorosis; I, plant that received 2 grams $Al_2(SO_4)_3$ showing no frenching. Faint chlorosis appeared on plant I 3 weeks after plants were photographed.

TABLE 4.—*Effect of aluminum sulphate on the development of frenching of tobacco in field soil*

Group	$Al_2(SO_4)_3$ (1 gm./pot) added at weekly intervals for: (weeks)	Number of plants	Number of plants frenched after:						Records after 70 days		
			30 days	35 days	44 days	54 days	60 days	70 days	Soil reaction (pH)	Green weight of plants (gm.)	Remarks
G	0	5	1	5	5	5	5	5	6.80	8.4	Severe rosette
H	1	5			2	5	5	5	6.57	9.3	Chlorosis
I	2	5					1	2	6.45	11.6	Slight chlorosis on 2 plants, rest healthy
J	3	5						1	6.40	7.8	Healthy
K	4	5						0	6.25	7.1	Healthy
L	5	5						0	6.15	5.8	Healthy

intervals to young seedlings grown in soil are recorded in table 4. These data indicate that the doses of $Al_2(SO_4)_3$ added greatly retarded the development of frenching. Figure 2 shows a plant from each of groups G, H, and I, as it appeared 42 days after potting. The extent to which $Al_2(SO_4)_3$ retarded frenching varied with the number of weekly treatments. Thus, with only one treatment, the first appearance of the disease was delayed nearly 2 weeks. With 2 treatments at weekly intervals, disease symptoms did not appear till 4 weeks after those in the check pots. With 3 treatments, only 1 plant out of 5 developed frenching within 10 weeks. The data in table 4 also show that the addition of $Al_2(SO_4)_3$ lowered slightly the reaction of the soil. $Al_2(SO_4)_3$ had only a slight depressing influence on growth. The apparent increase in growth with small amounts of $Al_2(SO_4)_3$, as shown in table 4, was due to the stunting that accompanies the development of frenching.

The addition of $Al_2(SO_4)_3$ was beneficial also in bringing about recovery from the frenched condition. In this experiment, the data from which are given in table 5, all of the plants were frenched before the first addition of $Al_2(SO_4)_3$ was made. Recovery was partial when only 1 or 2 grams of $Al_2(SO_4)_3$ were added per pot, but was complete when 3 grams were added in 1-gram quantities at weekly intervals. The degree to which $Al_2(SO_4)_3$ promoted recovery is shown by the plants in figure 3, photographed 48 days after first treatment. Thus, aluminum was effective not only in preventing the development of frenching, but also in bringing about recovery after the plants had become diseased. Similar results were obtained when $AlCl_3$ was substituted for $Al_2(SO_4)_3$.

To test the effect of aluminum and copper on the plant itself, young tobacco seedlings were potted in soil in 2-inch pots placed in glass saucers that were kept filled with water. After some 3 weeks all the plants became frenched; the roots by this time were extending down through the hole in the bottom of the pot. The pots were then suspended in glass tumblers. When the extending roots were submerged in a dilute nitrogen solution, the plants recovered. However, when the roots were submerged in a dilute solution of $Al_2(SO_4)_3$ or $CuSO_4$, recovery was only partial.

Inasmuch as frenching failed to develop on plants grown in sand and water cultures without the addition of both copper and aluminum, it is probable that the cause of frenching cannot be considered as a deficiency of either of these two elements, even though they had a pronounced action on the disease. The beneficial effect obtained by adding $Al_2(SO_4)_3$ or $CuSO_4$ to the soil probably was due to some change in the soil complex, rather than to any direct effect of the aluminum or copper on the plant itself.

These experiments on mineral deficiencies indicate definitely that frenching is not caused by a deficiency of nitrogen, phosphorus, potassium, calcium,

TABLE 5.—*Recovery from frenching by the addition of aluminum sulphate to the soil*

Group	$Al_2(SO_4)_3$ (1 gm./pot) added at weekly intervals for: (weeks)	Number of plants	Number of plants frenched after:						Records after 48 days		
			13 days	18 days	23 days	28 days	33 days	48 days	Green weight (gm.)	Soil reaction (pH)	Remarks
M	0	10	10	10	10	10	10	10	4.8	7.10	Severe rosette
N	1	10	10	10	10	10	10	10	6.1	7.05	Chlorosis
O	2	10	9	9	7	5	5	4	6.1	6.85	Faint chlorosis on 4 plants, rest healthy
P	3	10	9	6	4	2	0	0	5.9	6.70	Healthy

Photographed by J. A. Carlile

FIG. 3. Plants showing effect of aluminum sulphate on recovery from frenching. All plants showed frenching before experiment was started. M, plant that received no $Al_2(SO_4)_3$ showing severe frenching; N, plant showing partial recovery after receiving 1 gram $Al_2(SO_4)_3$; O, plant showing complete recovery after receiving 2 grams $Al_2(SO_4)_3$ at weekly intervals.

magnesium, sulphur, iron, boron, or manganese. Since these elements are the only mineral elements commonly considered as essential to plant growth, it is probable that frenching is not directly caused by a deficiency of any mineral nutrient.

Toxicity Studies. Since it was impossible to demonstrate that a deficiency of any of the essential elements is the cause of frenching, the next step was to determine if frenching is a toxicity disease. Experiments were carried out in which such factors as soil complex and mineral nutrition were eliminated as far as possible. In the first experiment seedlings were grown in washed quartz sand in 4-inch pots placed in saucers. One set of seedlings was watered with tap water and a similar set watered with a water extract of field soil. This extract was prepared as follows. Approximately 7 kilograms of soil were placed in a 5-gallon carboy and mixed thoroughly with about 15 liters of distilled water. When the soil had settled to the bottom of the carboy, the liquid was siphoned off and used in watering the plants. The plants watered with soil extract became frenched in about 7 weeks, whereas those watered with tap water were still healthy when the experiment was discontinued 5 weeks later.

In another experiment plants were grown in sand with various proportions of field soil. The experimental data are given in table 6. These

TABLE 6.—*The incidence of frenching in sand mixtures containing very small amounts of field soil*

Proportion of field soil added to sand		Number of plants	Number of plants frenched after:							
Per cent	Parts per thousand		3 weeks	4 weeks	5 weeks	6 weeks	7 weeks	8 weeks	9 weeks	23 weeks
0.0	0.0	5	0	0	0	0	0	0	0	0
0.05	0.5	5	0	0	2	3	3	4	5	
0.5	5.0	5	0	0	1	3	3	5		
0.75	7.5	5	0	0	2	5				
1.0	10.0	5	0	1	2	5				
10.0	100.0	5	4	5						

results show that it is possible to bring about the development of frenching in plants grown in quartz sand by the addition of as little as 1 part of field soil to 2000 parts of sand. Plants grown in sand to which no soil was added remained healthy and showed no symptoms of frenching.

It was later found possible to produce mild symptoms of frenching in plants grown in washed quartz sand without the addition of any soil whatsoever. Plants were grown in quartz sand in 4-inch porous clay pots placed in saucers kept partly filled with tap water from a deep well. At the end

of 5 months the plants, still healthy, were practically dormant, and were replaced by new seedlings. This second set of plants showed the chlorotic stage of frenching within 2 months after potting. These results may be explained as follows. The toxic material probably was present in the tap water in extremely small concentrations. With repeated watering of the plants from the bottom of the pots and continual evaporation of water from the pot and sand, this toxic material gradually accumulated in the sand. By this time the first set of plants had become nearly dormant and showed no evidence of toxicity. However, when new plants were planted in this sand and new growth started, the toxicity was made evident by the typical chlorosis characteristic of frenching in the tip leaves.

The 3 experiments described above would indicate that frenching is a toxicity disease rather than a mineral deficiency disease. The toxic principle was apparently added in the first experiment with the soil extract and in the second experiment with the trace of soil. Further experiments to determine the nature of the toxic principle are under way.

DISCUSSION

Although the experimental evidence presented in this paper indicates that frenching is a toxicity disease, this cannot be considered as conclusively established until the actual cause of the disease has been demonstrated. It is possible that the deficiency of some element, not recognized as essential to growth but nevertheless required in minute amounts and present as an impurity, may be the cause of frenching.

The experimental data have given no indication as to how the plant is affected by the causal agent of frenching. Therefore, it is difficult to explain how nitrogen, aluminum, or copper acts in preventing the development of frenching. These minerals may act directly on a toxic principle or indirectly by affecting some condition necessary for the liberation of such a principle. Soil texture, soil aeration, soil reaction, and mineral plant nutrition were found to be important secondary factors capable of modifying the incidence of the disease.

Further work may lead to a satisfactory explanation of the high susceptibility of certain Nicotiana species and the low susceptibiilty of many other species grown under similar conditions. It is possible that the Nicotiana species affected may be highly sensitive to some toxic element, since it is well known that plants differ in their tolerance of poisons.

The experimental evidence does not permit the formulation of any definite hypothesis as to the cause of frenching. However, the evidence does reveal some facts concerning the nature of the disease and the means by which it may be controlled.

SUMMARY

In the genus Nicotiana frenching developed severely in *N. alata, N. langsdorffii, N. longiflora, N. rustica, N. sanderae, N. sylvestris,* and in 16 varieties of *N. tabacum,* but not in 12 other species of Nicotiana grown under similar conditions. Of the 26 other solanaceous and non-solanaceous species tested, only *Datura stramonium, Lycopersicon esculentum,* and *Petunia hybrida* showed chlorosis characteristic of frenching.

Frenching in tobacco in a greenhouse was controlled by soil composting, the addition of peat, repeated applications of a nitrogenous fertilizer, and by several applications of a dilute solution of copper sulphate or aluminum sulphate.

No association was found between frenching and any pathogenic organism.

Experiments on the deficiency of each of the elements essential to plant growth failed to produce symptoms that resembled those of frenching.

Frenching was produced in plants grown in sand by the addition at daily intervals of a water extract of field soil. It also was produced by adding as little as 1 part of field soil to 2000 parts of sand. In young seedlings, in sand, it was produced by the addition of tap water from a deep well, over a long period of time.

The experimental evidence indicates that frenching probably is not a mineral-deficiency disease, but a toxicity disease produced by some toxic principle that is present in certain soils and that exerts its toxic action only under definite environmental conditions.

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A STUDY OF COALESCING HAPLOID PUSTULES IN PUCCINIA HELIANTHI[1]

A. M. BROWN[2]

(Accepted for publication February 18, 1935)

INTRODUCTION

It was shown by Craigie (5) that, in *Puccinia helianthi* Schw., when two haploid pustules of opposite sex coalesce, aecia develop in the compound pustule thus formed. He drew the theoretical deduction that hyphal fusions occur between the two mycelia and give rise to the diploid condition in which a nucleus of one sex is associated in conjugate relationship with a nucleus of the opposite sex. Somewhat later, however, he (6) discovered that the transfer of pycnial nectar from a haploid pustule of one sex to the pycnia of a haploid pustule of the opposite sex initiated the production of aecia in the pustule receiving the nectar.

The latter discovery raises the question as to whether or not the process of diploidisation can be initiated in *Puccinia helianthi,* or in any other similar heterothallic rust, by hyphal fusions between two haploid mycelia of opposite sex. In Craigie's early experiments, there was the possibility that the coalescence of pustules of opposite sex might have afforded an opportunity for an intermingling of their nectar. If that happened, the aecia might have arisen as a result of the functioning of the pycniospores, rather than as a result of hyphal fusions. To obtain further information concerning the point in question, an experiment was carried out in which precautions were taken to prevent any interflow of the pycnial nectar of coalescing pustules, or any accidental transference of nectar from one pustule to another.

In the Hymenomycetes, it is now well-established that the diplophase follows the union of paired haploid mycelia of opposite sex; and Buller (3 and 4) has shown that, when a haploid and a diploid culture of *Coprinus lagopus* coalesce, the mycelium of the former becomes diploidised. A similar result was obtained by Dickson (7) with *Coprinus sphaerosporus.*

Brown (2) found that when a haploid and a diploid (uredial) pustule of *Puccinia helianthi* coalesced, the haploid pustule became diploid and produced aecia. As pycnia are entirely absent from uredial pustules and as precautions were taken in the experiment to prevent any fortuitous transference by insects of pycnial nectar from one haploid pustule to

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another, no other conclusion seems possible but that the diplophase arose as a result of the interaction of the haploid and diploid mycelia. In a cytological study of heterothallism in *Puccinia coronata* Corda, Allen (1) mentions finding a hyphal fusion (illustrated in Plate 11, B) that she suggests may indicate the origin of the diplophase by hyphal fusions between haploid mycelia.

EXPERIMENTAL METHODS AND RESULTS

Haploid pustules of *Puccinia helianthi* were obtained by sowing sporidia from germinating teliospores on seedling plants of the cultivated sunflower, *Helianthus annuus L.* When the pustules appeared a careful inspection of them was made to distinguish as far as possible those of monosporidial origin from those of bisporidial origin. Such an inspection is fairly successful if made when the tiny pustule first excretes a minute globule of nectar. Among those pustules that appeared to be monosporidial, only those were selected that occurred in pairs, and among the pairs only those in which the two components of the pair were separated from each other by a distance of from 2 to 4 millimeters. Care was taken to protect the plants bearing the pustules from any agency that might accidentally intermix the pycnial nectar.

As a further precaution that all the pustules used in the experiment were haploid, the pustules were allowed to develop until they were 2 weeks or more old. If any individual pustule was of bisporidial origin and consisted of mycelia of opposite sex, it would most probably have produced aecia by the time it had reached this age. Only those pairs in which the two individual pustules were at this time still separated by a narrow strip of green tissue, or slightly chlorotic tissue in which no pycnia had yet developed, were included in the experiment proper.

In order to prevent the spontaneous intermixing of the nectar of each pair of pustules when later the two components would coalesce to form a compound pustule, a barrier was placed on the upper leaf surface between the two pustules of each pair. For this purpose, Eastman's Opaque No. 1 preparation was found quite satisfactory. It adhered readily to the leaf, dried quickly, and caused no perceptible injury. The barrier, or wall, was placed at right angles to a line passing through the centres of the two pustules comprising each pair and extended sufficiently far on either side of this line to assure its always exceeding any diameter that the pustules might later attain. The experiment was carried out during the winter of 1933–34. At this season of the year in Winnipeg the days are short and the light intensity is rather low, conditions under which the production of nectar is scanty and confined mostly to the central part of the pustule. The plants at this time of the year are almost free from insects. Hence

the possibility of any intermixing of pycnial nectar was practically eliminated.

A total of 288 pairs of pustules were included in the experiment. The 2 pustules of each pair were separated by a barrier of Opaque, as described above. Finally, they coalesced. The barrier appeared to have no adverse effect on the growth of the pustules: the radial expansion of the pustules, as observed on the under side of the leaves, was quite uniform. Of the 288 compound pustules that arose through the coalescence of the individual pairs of simple pustules, 110 produced aecia.

In the compound pustule that produced aecia, it almost invariably happened that aecia appeared in one component first and afterwards in the other component, aecial production being usually completed in the one before it became evident in the other (Fig. 1, A). In both components,

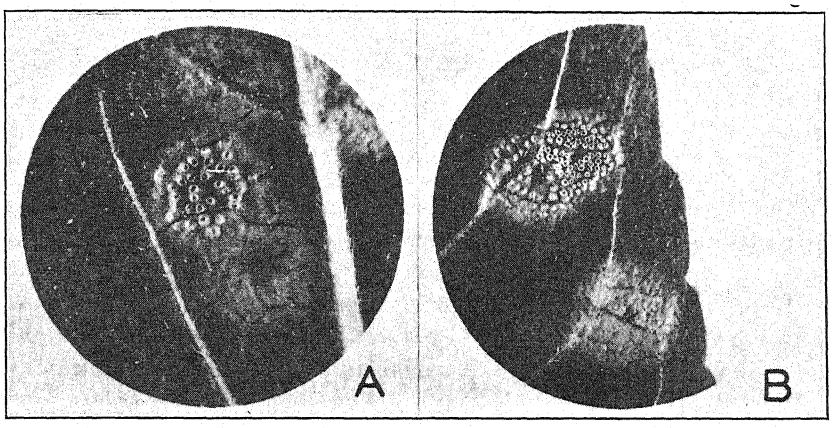


FIG. 1. A. Compound pustules of *Puccinia helianthi*. Aecia have formed in one component, but not in the other. ×5. B. Simple and compound pustules of *P. helianthi*. In one component of the compound pustule, the aecia are well developed, whereas in the other, the aecia are only beginning to appear. ×3.

aecia developed first, although at different times, in the areas adjacent to the line of fusion and latest in the areas most remote from that line. Figure 1, B shows that in one of the components the production of aecia is about completed, and that in the other component the development of aecia has progressed farthest near the line of fusion. There can be little possible doubt that, under the conditions of this experiment, the diploidisation process originates in the areas where the hyphae of the two mycelia first intermingle and that this process, as a rule, runs its course in one component before it makes any noticeable progress in the other.

The progressive diploidisation of one component of a compound pustule,

beginning in the region of coalescence and extending outward from this region to the opposite side of the component is further illustrated in figure 2, A, which shows a photomicrograph of a portion of a section cut parallel

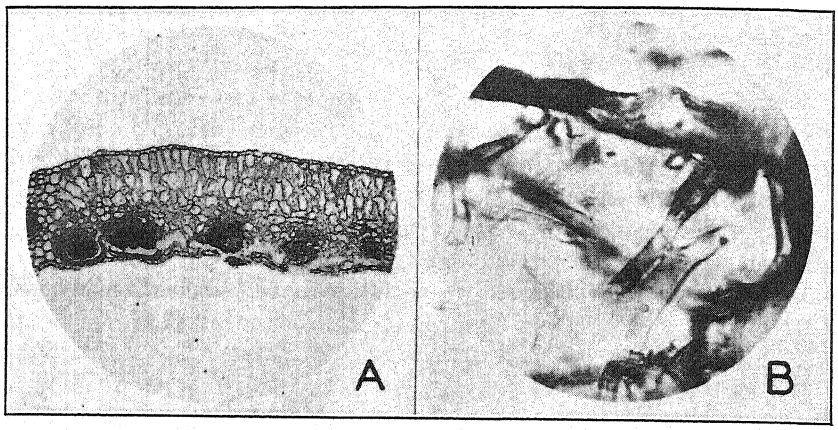


FIG. 2. A. Cross section through one component of a compound pustule of *Puccinia helianthi*. Explanation in the text. ×25. B. Hyphal fusion found in the region of coalescence of a compound pustule of *P. helianthi*. ×about 460.

to a line joining the centres of two pustules that had coalesced. A macroscopic examination of the under surface of the compound pustule, made just before the material was placed in a fixing solution, showed that aecia were beginning to develop in one of the components but not in the other. The region of coalescence is just to the left of the part shown in the photograph. It will be seen that the 2 neighbouring aecia on the left are about equally well developed and that both are producing chains of aeciospores (represented by the deeply-stained areas). In the third aecium the left-hand portion has become diploid and this portion is proceeding with the formation of aeciospores, although progress in this operation is less advanced than in the 2 aecia on the left. The remainder of the third aecium is still haploid, as are also the 2 aecial primordia still further to the right.

In view of the precautions taken in this experiment to guard against the transference of pycnial nectar from one pustule to another, the results obtained indicate that when 2 haploid pustules of opposite sex coalesce, they are able to diploidise each other without the intervention of pycniospores. It would appear that the interchange of nuclei must take place through hyphal fusions. In the region of coalescence, hyphal fusions have been found in compound pustules that were beginning to produce aecia. How many may occur in one compound pustule is not known, but at least one has been found in each of several compound pustules that were sectioned. Such a hyphal fusion is shown in figure 2, B. But no definite evidence

of nuclear migration was observed in any of the different fusions examined, and moreover, it was impossible to determine whether the fusions had occurred between 2 hyphae of the same mycelium, or between a hypha belonging to one mycelium and one belonging to the other mycelium.

DISCUSSION

The occurrence of aecia in one component of a compound pustule earlier than in the other component is a phenomenon that lends itself to speculation, but one for which it would seem difficult to determine the exact cause. As it occurs with such marked regularity, there must be some cause of its occurrence. One would suspect that when the two mycelia meet there would be ample opportunity for several, or even many, hyphal fusions to occur, and, therefore, opportunity for the diploidisation of the 2 components to take place simultaneously. This however very rarely happened. It may be that it is just a matter of chance as to which component first becomes diploidised and produces aecia. If that is the case, and on the assumption that only one hyphal fusion takes place, it may be supposed that a nucleus of one mycelium on entering the other mycelium proceeds rapidly to diploidise the latter mycelium. The formation of aecia follows close upon the advance (across the component) of the diploid condition. Such a sequence of events may so affect the physiological balance of the mycelium undergoing diploidisation that, until an approach to physiological equilibrium (complete or nearly complete diploidisation) in it is restored, no nucleus can pass over to the other mycelium to initiate the diploidisation of it. On the assumpton that 2 or more hyphal fusions occur and that 2 or more nuclei pass out from their own mycelium into the other mycelium, the movement must be in one direction first, namely, toward the mycelium that first becomes diploidised, and later towards the other mycelium; for, if nuclei passed in both directions, it is difficult to see why both mycelia do not become diploidised simultaneously.

On the other hand, it may be that it is not a matter of chance as to which component becomes diploidised first. There is the possibility that in compound pustules of which the components are of opposite sex, diploidisation regularly occurs first in those components belonging to one particular sex, for instance, in all the (+) components. Dickson (7) found that, when he mated 8 haploid strains of *Coprinus sphaerosporus,* not only did paired mycelia of opposite sex on uniting diploidise each other, but that, in general, the diploidisation was effected several days earlier in one member of each reacting pair than in the other. Also, he found that the strains first diploidised belonged to one sex, and could be distinguished from the other strains by their thinner aerial mycelium and their slower growth rate. The work reported in the present paper was done prior to the publication of

Dickson's paper, and no particular attention was paid to the rate of increase in pustule size, the character that in *Puccinia helianthi* approaches most nearly to, but probably cannot be considered a substitute for, rate of mycelial growth; but if the components that first produced aecia were regularly of less diameter than their respective mates, it is unlikely, although not impossible, that this difference passed unnoticed. Of course, the point can be decided only by someone making the necessary measurements.

Unless it be found that haploid pustules of one sex consistently increase in size more rapidly than haploid pustules of the opposite sex, the determination of which pustules are of one sex and which of the opposite sex will prove a very difficult matter, as in all other macroscopic characteristics pustules of both sexes are, as far as known, identical; and biochemical methods of differentiation would encounter very great difficulties, owing to the limited size of the pustules and to the fact that the organism can develop only in intimate association with the tissues of the host.

SUMMARY

Evidence is presented indicating that, in *Puccinia helianthi* Schw., when 2 haploid pustules of opposite sex coalesce to form 1 compound pustule, diploidisation of the mycelia of the components of the compound pustule can be effected without the intervention of pycniospores. The diploidisation process is initiated, apparently, by hyphal fusions and it is completed, or almost completed, in one component before it is begun in the other component.

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STUDIES ON SEVEN DIFFERENTIATING CHARACTERISTICS OF TWO PHYSIOLOGIC FORMS OF TILLETIA TRITICI[1]

C. S. HOLTON

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INTRODUCTION

Physiologic forms of *Tilletia tritici* (Bjerk.) Wint. are identified and separated primarily on the basis of their pathogenic properties. This, however, does not necessarily mean that they are alike in all other respects. Differences between physiologic forms of *T. tritici* with respect to smut-ball type, prominence of the spore-wall reticulations, and degree of stunting of the plants have been noted by various workers, while other reports (4, 6) indicate that strains of the bunt organisms do not differ consistently in cultural characteristics. Bressman (2), Smith (11), and Brentzel (1) have reported differences in physiologic forms of *T. tritici* with respect to type of smut balls produced, and Rodenhiser (9) found that certain forms of the bunt organisms stunt the plants more than do others. It is evident, therefore, that physiologic forms of *T. tritici* may differ in several respects other than pathogenicity. As yet, however, little consideration has been given to the significance of these differences as criteria in establishing the identity of physiologic forms of *T. tritici*.

In 1933 the writer (5) reported that 2 physiologic forms of *Tilletia tritici* on the variety Hohenheimer (C. I. 11458) were distinguishable on the basis of pathogenicity, spore-ball size and shape, spore-wall reticulations, or rate of chlamydospore germination. Further studies have shown that these 2 forms of *T. tritici* differ also in certain other characteristics. The difference in rate of chlamydospore germination reported previously has been less constant than preliminary tests had indicated. Therefore, it is not included in this discussion.

RESULTS

In the physiologic-form smut nursery at Pullman, Washington, in 1931, a form of *Tilletia tritici* collected at Pullman produced large, elongate smut balls on the variety Hohenheimer, while another form, from Lind, Wash., produced small, somewhat rounded smut balls on the same variety (Fig. 1). According to the results in 1931, there was no significant difference between the pathogenic properties of these 2 forms of *T. tritici*. Nevertheless, they were considered as distinctly different strains on the

[1] Cooperative investigations between the Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture, and the Washington, Oregon, and Idaho Agricultural Experiment Stations.

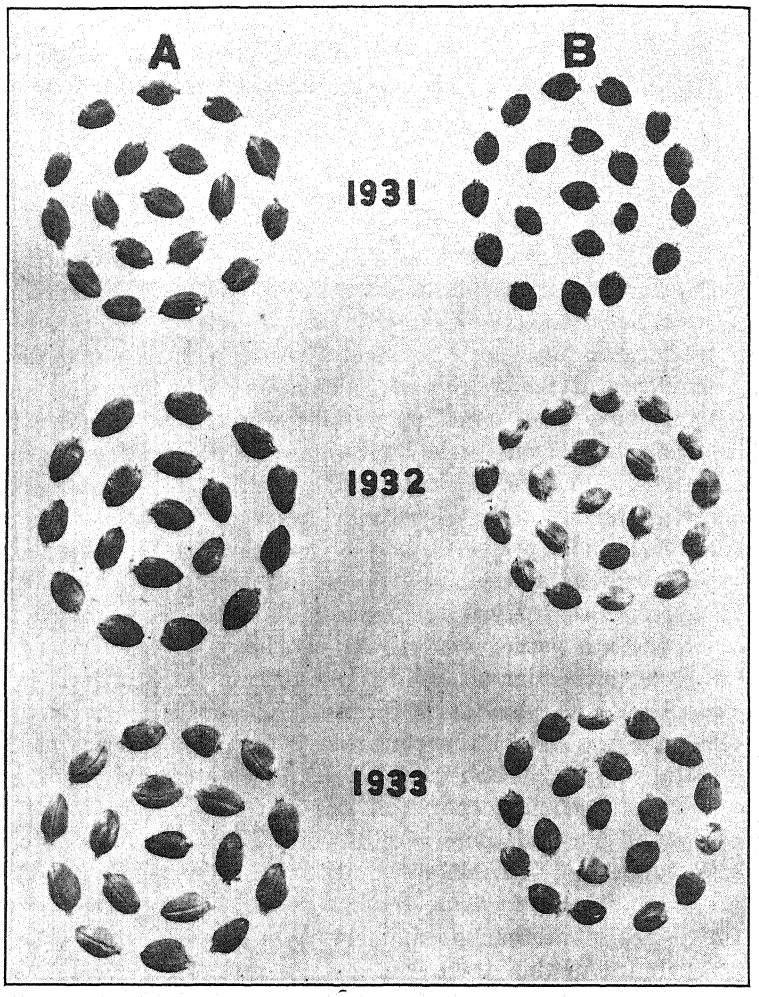


FIG. 1. Types of smut balls produced on Hohenheimer by 2 physiologic forms of *Tilletia tritici*. A. Pullman form. B. Lind form.

sole basis of difference in smut-ball type. Additional tests were made at Pullman, Wash., and at Pendleton and Corvallis, Oreg., to determine whether this difference would remain constant over a period of years and under different environmental conditions. It was considered probable, also, that a difference in pathogenicity might be exhibited in these more extensive tests. The results of 3 years' tests, 1932–34, indicate clearly that the 2 forms are distinctly different pathogenically (Table 1). Based on a 3-year average, the Pullman form produced 33.0 per cent smut on Hohenheimer as compared to 81.0 per cent produced by the Lind form on

TABLE 1.—*Differentiating characters of 2 physiologic forms of Tilletia tritici on the variety Hohenheimer*

Form	Smut	Smut-ball size and shape	Spore-wall reticulations	Mean diameter of spores	Mean length of promycelia	Mean height of culms		Nuclear behavior
						Smut-free	Smutted	
	Per cent			μ	μ	*Cm.*[a]	*Cm.*[a]	
Pullman, Wash.	33.0	Large and elongate	Faint	17.85 ± 0.03	72.62 ± 1.60	118.48 ± 0.44	99.80 ± 0.52	Nuclei scattered
Lind, Wash.	81.0	Small and rounded	Prominent	19.31 ± 0.04	21.45 ± 0.39	118.48 ± 0.44	92.84 ± 0.43	Nuclei in a group

[a] Measurements on the height of culms were made on the variety Hosar.

the same variety. Approximately the same difference in pathogenicity of the 2 forms was apparent on Hosar (C. I. 10067), but the other varieties tested, except Hybrid 128 (C. I. 4512), were highly resistant. The differences in smut-ball type were constant over a 4-year period, 1931–34, as shown for the first 3 years at Pullman, in figure 1. Similar differences in pathogenicity and spore-ball type were observed at Pendleton, Oregon.

The results of studies to ascertain in how many ways these 2 Hohenheimer forms of *Tilletia tritici* differ are summarized in table 1. All studies were made with material taken from Hohenheimer, except for the study of the relative degree of stunting of the plants, in which the variety Hosar was used. It was established, as indicated in table 1, that there are 7 ways in which these 2 forms may be distinguished from each other. As reported in 1933 (5), there is, in addition to pathogenicity and spore-ball characteristics, a distinct difference in the prominence of the spore-wall reticulations. The reticulations on the chlamydospores of the Pullman form are relatively faint or inconspicuous in contrast to the very prominently reticulated walls of the spores of the Lind form, which give the spores of this form a somewhat spiny appearance. Brentzel (1) has reported similar observations on 2 forms of *T. tritici* on Ceres wheat.

A difference in the size of the chlamydospores of the two forms of *Tilletia tritici* on Hohenheimer also was established. Measurements were made of 252 chlamydospores of each form, and it was found that the mean diameter of the spores of the Pullman form averaged $17.85 \pm 0.03\ \mu$ as compared to $19.31 \pm 0.04\ \mu$ for the Lind form (Table 1). These figures show a significant difference of $1.46 \pm 0.05\ \mu$ in the diameter of the chlamydospores of the 2 forms. Similarly, measurements were made of 100 promycelia of each of the 2 forms, and it was found that the mean length of the promycelia of the Pullman form was $72.62 \pm 1.60\ \mu$ as compared to $21.45 \pm .39\ \mu$ for the promycelia of the Lind form. This shows a difference in length of the promycelia of $51.17 \pm 1.65\ \mu$, which is 31 times as great as the probable error of the difference. The measurements were made from the points of attachment of the promycelium to the chlamydospore and the primary sporidia to the promycelial tip. It also was found that there was a difference in the degree of stunting of the plant caused by these 2 forms. The mean height of 92 smut-free culms was 118.48 ± 0.44 cm., as compared to a mean height of 99.80 ± 0.52 cm. for 80 culms infected with the Pullman form and 92.84 ± 0.43 cm. for 176 culms infected with the Lind form. These figures show that there is a definite stunting of the plants by both forms, the difference in height between the smut-free culms and culms smutted with the Pullman form being 17.41 ± 1.68 cm., while the difference in height between the smut-free culms and culms smutted with the Lind form was even greater, 24.31 ± 1.61 cm. The difference

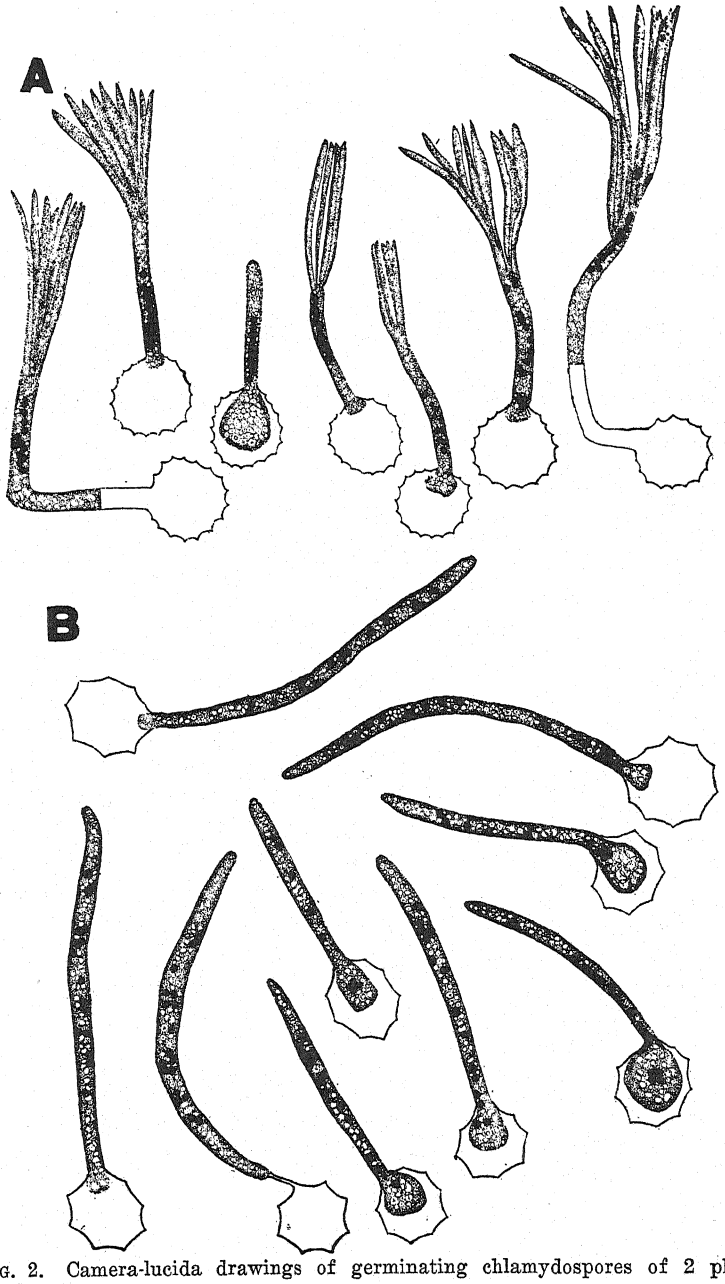


FIG. 2. Camera-lucida drawings of germinating chlamydospores of 2 physiologic forms of *Tilletia tritici*, showing the difference in nuclear distribution in the promycelium. A. Lind form. B. Pullman form. ×830.

in the height of the culms infected with the Pullman form and the Lind form was 6.89 ± 0.68 cm., showing that the Lind form causes a greater degree of stunting in the variety Hosar than the Pullman form. Field observations indicate rather definitely that these 2 forms of *T. tritici* differ in this respect also on Hohenheimer, but actual measurements on this variety have not been made.

A difference in the nuclear behavior at the time of germination of the chlamydospores of the 2 forms of *Tilletia tritici* also was established. As indicated in table 1, the nuclei of the Pullman form were scattered, while those of the Lind form remained in a group. Aside from its significance as a differentiating character between these 2 forms of *T. tritici,* this difference in nuclear phenomenon may help to explain the conflicting view-points on nuclear behavior in *Tilletia spp.* expressed by earlier workers. Dastur (3) observed that the undivided spore nucleus of *T. tritici* passed into the single-cell promycelium before the first and subsequent divisions occurred. On the other hand, Paravicini (7) had previously reported that the first division takes place within the spore, and one nucleus passes into the promycelium, the other remaining in the spore. Later, Rawitscher (8) maintained that all nuclear divisions take place within the spore during germination and that, when the promycelium is produced, as many as 16 nuclei may migrate into it. The type of nuclear behavior observed in the 2 physiologic forms of *T. tritici* described in this paper is similar to that observed by Rawitscher (8). All divisions of the nuclei take place within the spore and later the nuclei migrate into the promycelium. However, the distribution of the nuclei in the spore and promycelium is not the same in the 2 forms. As pointed out previously, the nuclei in the Pullman form were scattered, while those in the Lind form were grouped. This difference was apparent while the nuclei were still in the spore, as shown in figure 2, and it continued to persist during and after the process of migration into the promycelium, as shown in figure 3. Certain exceptions to the general rule were observed, and some of these exceptions are shown in figures 2 and 3. Exceptions are expected, howver, but they do not invalidate the general rule. In view of the difference in the nuclear behavior in the 2 forms of *T. tritici* described herein, it seems very probable that the opposing view-points of Paravicini (7), Dastur (3), and Rawitscher (8) were based on studies of different physiologic forms.

SUMMARY AND CONCLUSIONS

Seven differentiating characteristics were established between 2 physiologic forms of *Tilletia tritici* that attack the variety Hohenheimer (C. I. 11458). Aside from differing in pathogenic properties on Hohenheimer, the 2 forms differ also in type of smut ball, prominence of the spore-wall

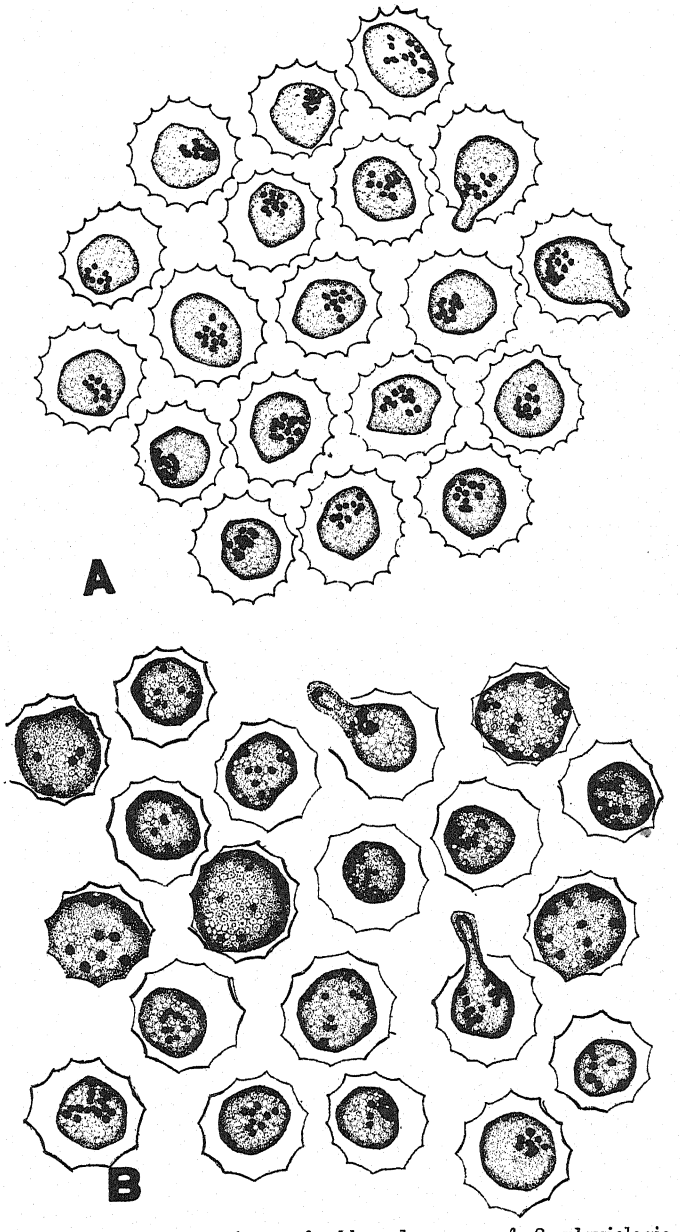


FIG. 3. Camera-lucida drawings of chlamydospores of 2 physiologic forms of *Tilletia tritici*, showing the difference in nuclear arrangement in early stages of germination. A. Lind form. B. Pullman form. ×1850.

reticulations, size of the spores, length of the promycelia, and the nuclear behavior during germination of the chlamydospores. On the variety Hosar (C. I. 10067), they differ also in the degree of stunting of the host plants. This is the largest number of characteristics in which 2 physiologic forms of *T. tritici* have been found to differ. Three of these differences—size of chlamydospores, length of promycelium, and nuclear behavior—apparently have not been reported previously. While it is recognized that, from an economic point of view, a difference in the pathogenicity of physiologic *forms of T. tritici* and *T. levis* is more important than other differences, it also is recognized that, in view of Smith's (10) results, pathogenic properties may vary under different environmental conditions. For this reason, it cannot be expected that the pathogenic reaction of physiologic forms of these organisms will be the same in every locality. Therefore, in view of the great need for a uniform system of identifying and numbering physiologic forms of *T. tritici* and *T. levis*, perhaps it would be wise to take into account, because of their possible usefulness in establishing such a system, differences (other than pathogenicity) between forms.

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EFFECT OF CROWN GALL, HAIRY ROOT, AND WOOLLY APHIDS ON APPLE TREES IN THE ORCHARD

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The losses to individual apple nurseries in Tennessee during the early twenties, due to the culling of trees on which had developed crown gall, hairy root, and woolly-aphid knots, often amounted to 50 per cent—sometimes running as high as 90 per cent—of the total number of trees produced. The situation called for two lines of study: one to find, as quickly as possible, effective, practical methods of control; the other to determine by actual orchard tests just how important it is to avoid, in Tennessee, the planting of affected trees. This paper is a report of the latter study, in which tests were made of the effects of the troubles on trees in the orchard under Tennessee conditions.

Such tests were important, first, because most workers, especially in the northeastern States, were convinced that crown gall was of no significance in the orchard; and, second, because many of our nurserymen reported apparently definite observations, and what appeared to be special tests, showing that culled nursery trees, seriously affected with one of the troubles in question, produced as well in the orchard as any so-called healthy trees. The nurserymen did not believe it necessary to destroy the culled trees.

To obtain experimental data on this much disputed question that would apply to conditions in Tennessee, a test orchard was set out on the West Tennessee Experiment Station farm, at Jackson, early in the spring of 1923. The location had previously been set out to apple trees, most of which died or became undesirable, and the plot, therefore, was cleared prior to the setting of the test trees. Four times as many trees were set out as were expected to remain permanently in the orchard.

Twelve hundred trees were planted—the number of each variety being as follows:

Black Ben Davis	135
Delicious	80
Early Harvest	165
Horse	55
Kinnard	105
Mammoth Black Twig	80
Red June	70
Rome	55
Stayman	200
Wealthy	40

Winesap	120
Yellow Transparent	40
York Imperial	55
Total	1200

The trees were set in the rows according to variety. At the time of planting, 19.8 per cent had large crown galls, 11.5 per cent medium-size, and 9.5 per cent small crown galls; 20.1 per cent had extensive and 8.2 per cent slight development of hairy root; 9.3 per cent had extensive and 8.5 per cent slight development of woolly-aphid knots; and 13.1 per cent were apparently healthy. So far as diseases and injuries were concerned, the trees were planted at random. They were neither fumigated nor disinfected in any manner.

In February, 1924, one year after planting, 55 trees in permanent locations were found dead and were replaced with apparently healthy ones. The distribution of the dead trees according to their condition at planting time is shown in table 1.

TABLE 1.—*Distribution of dead trees in permanent locations, February, 1924, according to their condition at planting time*

Condition of tree at planting time, spring of 1923	Dead trees in permanent locations, February, 1924	
	Number	*Per cent*
Crown gall, large	18	32.6
" " medium	3	5.5
" " small	3	5.5
Hairy root, extensive	6	10.9
" " slight	5	9.1
Woolly-aphid knots, extensive	14	25.4
" " " slight	3	5.5
Apparently healthy	3	5.5

The number of trees recorded in table 1 is very small, and most of the figures, therefore, have little significance. They show, however, that the death rate was rather high—significantly so, it appears—among the trees that were severely affected with woolly aphids at planting time or had large crown galls, compared with those that were affected slightly or not at all.

In December, 1925, 3 seasons after the test trees were set out in the orchard, 595 of them, or nearly half the total number, were dug up in systematic order and their condition recorded, with the results shown in table 2.

With the exception of the data for the trees originally having medium-size crown galls, the figures presented in table 2 indicate that the death rate and health condition of the trees were in correlation with the condition

TABLE 2.—*Condition of trees dug up in December, 1925, three seasons after being set in test orchard, classified according to their condition at planting time*

Condition of tree at planting time, spring of 1923	Number of trees dug up	Condition of trees at digging time, December, 1925			
		Dead	With large crown gall	With poor or rotted roots	Poor roots and dead combined
		Per cent	*Per cent*	*Per cent*	*Per cent*
Crown gall, large	123	34.1	35.8	5.7	39.8
" " medium	66	9.1	37.9	6.1	15.2
" " small	50	18.0	28.0	14.0	32.0
Hairy root, extensive	134	18.6	31.4	17.9	36.5
" " slight	49	14.3	28.6	18.3	32.6
Woolly-aphid knots, extensive	33	54.6	12.1	3.1	57.7
" " " slight	79	20.2	15.2	7.6	27.6
Apparently healthy	61	4.9	21.3	6.6	11.5

of the trees at planting time. The highest death rate, 54.6 per cent, was for the trees originally showing extensive development of woolly-aphid knots;[1] and the next highest, 34.1 per cent, for those originally having large crown gall. The lowest death rate, 4.9 per cent, was for the trees that were apparently healthy at planting time. The remaining classes, except one originally with medium-sized crown gall, occupy about the same position—an intermediate between that of the trees extensively affected with woolly-aphid knots and with large crown gall, on the one hand, and trees apparently healthy on the other. The figures in the last column, showing percentage of the dead trees and those with poor or rotted roots combined, also appear to be significant. These figures indicate that the number of dead trees and trees with poor or rotted roots is in direct proportion to the degree of development of any of the troubles affecting the trees at planting time. The number is the highest for extensive woolly-aphid infestation, followed in order by large crown gall, and extensive hairy root development. The death rate for the trees apparently healthy at setting time is the lowest, and for all others—again except the group originally with medium-size crown gall —occupying an intermdiate one.

The exceptionally low figures for dead and poorly rooted trees in the medium-size crown-gall group can be explained by the chance location of most of the trees in soil unfavorable for rapid dying or for severe development of the trouble, or by the possibility that in this group were placed many trees not affected with true crown gall but with moderately developed

[1] If the trees with woolly-aphid knots had been effectively fumigated before planting, the results might have been different.

callus overgrowths. This might actually have happened, because at planting time no sharp distinction was made in the grading between crown gall and callus overgrowth, though the difference was known to the graders and was most likely observed, at least in the grading of trees with large galls. In any case, the exception shows that under the conditions of the experiment no special significance can be attached to small differences in all of the figures or to an isolated record, even with a markedly different figure.

In December, 1928, 6 seasons after the trees were planted, 157 of them were dug up, again in systematic order, and their general condition recorded, as shown in table 3.

TABLE 3.—*Condition of trees dug up in December, 1928, six seasons after being set in test orchard, classified according to their condition at planting time*

Condition of tree at planting time, spring of 1923	Number of trees dug up	Condition of trees at digging time, December, 1928				
		Very good	Good	Poor[a]	Dead	Dead and poor
		Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Crown gall, large	31	19.3	19.3	35.5	25.9	61.4
" " medium	25	20.0	44.0	28.0	8.0	36.0
" " small	30	13.3	50.0	23.4	13.3	36.7
Hairy root, extensive	29	13.8	51.7	13.8	20.7	34.5
" " slight	10	0.0	70.0	30.0	0.0	30.0
Woolly-aphid knots, extensive	12	25.0	8.3	16.7	50.0	66.7
Woolly-aphid knots, slight	7	43.4	28.6	14.0	14.0	28.0
Apparently healthy	13	61.5	23.1	7.7	7.7	15.4

[a] Trees with root system in such poor condition that they were expected to be dead within 2 or 3 years.

While the figures in table 3 are small and no special significance can be attached to any individual case, the general trend is indicative of certain correlations. It seems clear, for example, that the number of dead and "poor" trees combined is around 4 times as great for the trees originally with extensive woolly-aphid knots and with large crown galls, and twice as great for the other classes of affected trees, as for the originally healthy trees.

The trees that were not dug up in 1925 and 1928 were those planted mostly in permanent locations. These were left undisturbed except that now and then dead ones were replanted. The first record of such dead, replanted trees was made in 1924 and has been already presented here. The remaining records have not yet been completely analyzed, hence they are not available

for presentation at this time. The figures that are available, however, indicate the same trend as those discussed, namely, that the highest death rate was among the trees originally with extensive development of woolly-aphid knots and with large crown galls, and the lowest among the trees apparently healthy at planting time.

In conclusion, it may be stated that the test seems to indicate clearly that the death rate of apple trees in the orchard is correlated with, and proportional to, the development of crown gall, woolly-aphid knots, and, apparently, hairy root, on the trees used for the planting. The test shows clearly that it is not safe to plant such trees in the orchard. It must be understood, however, that the trees were neither fumigated nor disinfected in any manner before they were planted.

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DATES OF PRODUCTION OF THE DIFFERENT SPORE STAGES OF CRONARTIUM RIBICOLA IN THE PACIFIC NORTHWEST

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(Accepted for publication February 26, 1935)

White pine blister rust (*Cronartium ribicola* Fisch.) was first discovered in western North America in the vicinity of Vancouver, British Columbia, in the fall of 1921.[2] Following this discovery, investigations of the disease have been conducted annually in the West since 1922 by the Division of Forest Pathology, Portland, Oregon, for the purpose of accumulating facts on the behavior of the rust under the new host and environmental conditions pertinent to the development of control measures. One phase of these large-scale studies has been to secure data annually on the date of occurrence of the various spore stages in the life history of the rust on white pines and ribes.[3] These data, which are herein presented in tabular form (Tables 1 and 2), show some of the more important dates in the development of the white pine blister rust on the two different hosts, and their variation according to season and locality.

In tables 1 and 2 it will be noted that British Columbia has been first broadly divided into 2 regions—coastal and interior. Subdivisions of these 2 regions have then been made and the data grouped thereunder. The map (Fig. 1) shows regional divisions, subdivisions, and localities referred to in the tables. The dry belt includes that part of the interior region outside the range of *Pinus monticola* Dougl. The Canadian Pacific Railway has been used as the dividing line between the southern and northern dry-belt sections. All other regional subdivisions or localities mentioned are self-explanatory on the map. All subdvisions are based upon climatic, altitudinal, and vegetational differences between the particular sections of country in question. No data were obtained in the higher elevations of the

[1] Assistant Pathologists, Division of Forest Pathology, U. S. Bureau of Plant Industry, Portland, Oregon. The writers were assisted in the collection of data used in this paper by H. G. Lachmund and T. S. Buchanan, of the Division of Forest Pathology; by C. N. Partington, A. A. McCready, and W. F. Cummins, formerly of that Division; and by several members of the Division of Plant Disease Control, Spokane, Washington.

[2] Davidson, A. T. Report of the Proceedings, Third Western White Pine Blister Rust Conference, Portland, Oregon, Nov. 22, 23, 1923 (Mimeographed).

[3] The genus name Ribes is used in this paper to include both currants and gooseberries.

TABLE 1.—*Dates of aecial and pycnial development on white pines in different regions*[a]

Development on pines	Year	BRITISH COLUMBIA — Coastal Region: Near sea level Elevation 0-200'	Coastal Region: Garibaldi (Mile 22, PGE Ry.) Elevation 1100'	Coastal Region: Mile 72 PGE Ry. Elevation 1300'	Coastal Region: Prohibition Claim Plot and Tenquil Elevation 3000-5400'	Interior: Range of Pinus monticola Elevation 1150-2500'	UNITED STATES — Washington: Western Wash. Range of Pinus monticola Near sea level	Oregon: Rhododendron Elevation 1700'
First appearance of aecia	1922	April 1	.....	.....	.....	.....	.....	.....
	1923	March 17	Early Apr.	Early May	.....	.....	.....	.....
	1924	March 15	" "	" "	.....	Early May	.....	.....
	1925	March 11	" "	" "	.....	.....	.....	.....
	1926	March 9	" "	Mid-Apr.	.....	.....	Late Feb.	.....
	1927	Mid-Mar.	Mid-Apr.	.....	Mid-May	.....	.....	.....
	1928	" "	Early Apr.	.....	Late Apr.	Early Apr.	Early Mar.	.....
	1929	.....	.....	Late Apr.	Early May	Late Apr.	Apr. 7	Apr. 6
	1930	.....	Late Apr.	.....	.....	.....	Mar. 16	Early Apr.
	1931	.....	Late Mar.	.....	.....	Early Apr.	Mar. 1	Mid-Mar.
	1932	.....	Mid-Apr.	.....	.....	.....	Apr. 5	Late Mar.
	1933	.....	Late Apr.	.....	.....	Mid-Apr.	Early Apr.	Early Apr.
	1934	.....	Mid-Mar.	.....	.....	.....	Late Feb.	Late Feb.
Main period of aecial sporulation	1922	Mid-Apr. to mid-Aug.	.....	.....	.....	.....	.....	.....
	1923	Early Apr. to early June	Mid Apr. to early June	Mid May to late June	.....	.....	.....	.....
	1924	Early Apr. to mid-June	Mid-Apr. to late June	" "	.....	Mid-May to mid-July	.....	.....
	1925	Late Mar. to mid-June	Late Apr. to late June	Early May to late June	.....	.....	.....	.....
	1926	Late Mar. to early May	Mid-Apr. to mid-May	Late Apr. to lat: May	.....	Mid-May to late May	.....	.....
	1927	Mid-Apr. to mid-June	Late Apr. to late May	.....	Early June to early Aug.*	Late Apr. to early June	.....	.....
	1928	Late Mar. to early May	Mid-Apr. to mid-June	.....	Mid-May to early July	Early May to mid-June	.....	.....
	1929	Late Apr. to late May	Late Apr. to mid-June	Early May to early June	Late May to early July	Mid-May to mid-June	Early May to mid-June	.....
	1930	.....	Early May to mid-June	.....	.....	Late Apr. to early June	Late Apr. to late June	Mid-Apr. to mid-May
	1931	Early Apr. to mid-May	" "	.....	.....	" "	.....	Late Mar. to early May
	1932	.....	.....	.....	.....	Early May to late May	.....	Late Apr. to late May
	1933	.....	.....	.....	.....	Late Apr. to early June	Late Apr. to late June	Late Apr. to early June
	1934	.....	.....	.....	.....	.....	.....	Mid-Mar. to mid-Apr.
Maximum aecial sporulation	1922	Late May	Late June	Mid-July	.....	.....	.....	.....
	1923	Early May	Mid-May	Early June	.....	Late May	.....	.....
	1924	Late Apr.	Early May	Late May	.....	Early June	.....	.....
	1925	" "	" "	" "	.....	.....	.....	.....
	1926	Early May	Late Apr.	Mid-May	.....	.....	.....	.....
	1927	Mid-May	Early May	.....	Mid-July*	Mid-May	.....	.....
	1928	Early May	Late Apr.	.....	Mid-June	Late May	.....	.....
	1929	" "	Mid-May	Late May	" "	" "	Late May	.....
	1930	.....	" "	.....	.....	Early May	Mid-May	Early May
	1931	Late Apr.	" "	.....	.....	" "	.....	" "
	1932	.....	Early May	.....	.....	Mid-May	.....	" "
	1933	.....	.....	.....	.....	" "	Mid-May	" "
	1934	.....	.....	.....	.....	.....	.....	Early Apr.
First pycnial drops	1922	June 24	.....	.....	.....	.....	.....	.....
	1924	Apr. 21	.....	.....	.....	.....	.....	.....
	1925	Mid-May	.....	.....	.....	.....	.....	.....
	1926	" "	.....	.....	.....	.....	.....	.....
	1927	Early June	.....	.....	.....	.....	.....	.....
	1928	.....	.....	.....	.....	.....	May 30	.....
	1929	.....	.....	.....	.....	.....	June 19	.....
	1930	.....	June 22	.....	.....	June 24	.....	.....
	1931	.....	Early June	.....	.....	June 25	.....	.....
	1933	.....	.....	.....	.....	July 1	.....	.....
	1934	.....	.....	.....	.....	.....	.....	Late May
Main period of pycnial production	1922	Late June to early Aug.	.....	.....	.....	.....	.....	.....
	1924	Mid-June to early Sept.	Early July to late Aug.	.....	.....	.....	.....	.....
	1925	Mid-June to Early Aug.	.....	.....	.....	.....	.....	.....
	1930	.....	Late June to late Aug.	.....	.....	.....	.....	.....
	1931	.....	" " "	.....	.....	Early July to late Aug.	.....	.....
	1933	.....	.....	.....	.....	" "	.....	.....
	1934	.....	.....	.....	.....	.....	.....	Early June to early Aug.
Last pycnial drops	1924	Sept. 16	Late Sept.	Mid-Aug.	.....	.....	.....	.....
	1926	.....	.....	.....	.....	.....	Aug. 12	.....
	1931	.....	Oct. 29	.....	.....	Oct. 29	.....	Oct. 7
	1934	.....	.....	.....	.....	.....	.....	Sept. 28

[a] An asterisk (*) following a date or period in the table indicates that the data were secured on *Pinus albicaulis;* all other data were secured on *P. monticola.*

TABLE 2.—*Dates of uredinial and telial development on ribes in different regions*

DEVELOPMENT on RIBES	Year	BRITISH COLUMBIA							UNITED STATES
		Coastal Region				Interior Region			
		Near sea level Elevation 0-200'	Garibaldi (Mile 22, PGE) Elevation 1100'	Mile 72 PGE Ry. Elevation 1300'	Prohibition Claim Plot and Tenquil Elevation 3000-5400'	Range of Pinus monticola Elevation 1150-2500'	Northern dry belt Elevation 1000-3600'	Southern dry belt Elevation 1000-3600'	Western Washington Elevation 0-500'
First uredinia	1922	May 18	.....	Mid-July	.....	.....	.....	.....	.....
	1923	May 7	May 21	Mid-June	.....	June 16	.....	.....	.....
	1924	May 21	Early June	Late June	.....	June 13	.....	.....	.....
	1925	May 10	May 10	May 23	.....	.....	.....	.....	.....
	1926	Apr. 25	Apr. 27	Early May	.....	Late May	.....	.....	.....
	1927	May 22	May 22	May 26	.....	.....	.....	.....	.....
	1928	May 11	.....	May 15	.....	June 5	.....	.....	May 11
	1929	.....	May 28	.....	June 17	Mid-June	.....	.....	May 22
	1930	May 8	Mid-May	.....	.....	Early June	.....	.....	May 6
	1931	.....	.....	.....	.....	Late May	.....	.....	.....
	1932	.....	.....	.....	.....	Early June	.....	.....	.....
	1933	.....	.....	.....	.....	June 13	.....	.....	.....
	1934	.....	.....	.....	.....	Early June	.....	.....	.....
First telia	1922	June 16	Early July	.....	.....	.....	.....	.....	.....
	1923	June 3	Mid-June	.....	.....	July 9	.....	.....	.....
	1924	Mid-June	" "	Early July	.....	Early July	.....	.....	.....
	1925	Late May	Late May	" "	.....	.....	.....	.....	.....
	1926	Mid-May	May 15	May 26	.....	.....	.....	.....	.....
	1927	June 7	June 2	June 17	.....	.....	.....	.....	.....
	1928	.....	May 30	.....	.....	.....	.....	.....	June 7
	1929	.....	Late June	.....	.....	.....	.....	.....	" "
	1930	.....	Mid-June	.....	.....	June 24	.....	.....	.....
	1931	.....	.....	.....	.....	June 11	.....	.....	.....
	1932	.....	.....	.....	.....	Late June	.....	.....	.....
	1933	.....	.....	.....	.....	July 6	.....	.....	.....
	1934	.....	.....	.....	.....	Late June	.....	.....	.....
Peak of telial production	1922	Late Sept.	Early Sept.	Early Oct.	.....	Early Sept.	.....	.....	Late Oct.
	1923	Late July	Late July	Mid-Aug.	.....	Mid-Aug.	.....	Late Sept.	Early Oct.
	1924	Late Aug.	Mid-Aug.	Early Sept.	.....	Early Sept.	.....	Late Oct.	October
	1925	Late Sept.	Late Sept.	Early Oct.	.....	" "	Early Oct.	Late Sept.	.....
	1926	Late Aug.	Late July	Early Sept.	.....	Late Sept.	Mid-Sept.	Early Sept.	Early Aug.
	1927	Mid-Sept.	Mid-Aug.	Late Sept.	.....	Early Sept.	Early Sept.	Mid-Sept.	Late Aug.
	1928	Early Sept.	Late July	.....	Mid-Sept.	.....	Late Aug.	Late Aug.	Mid-Aug.
	1929	Mid-Sept.	Late Sept.	.....	Early Oct.	Late Aug.	Early Sept.	Early Sept.	.....
	1930	Early Sept.	Late Aug.	.....	Mid-Sept.	Early Aug.	" "	" "	.....
	1931	Late Aug.	Mid-Aug.	.....	.....	Late July	.....	.....	.....
	1932	.....	.....	.....	.....	Early Aug.	.....	.....	.....
	1933	.....	.....	.....	.....	Late Aug.	.....	.....	.....
	1934	.....	.....	.....	.....	Late July	.....	.....	.....

interior region where white-bark pine, *Pinus albicaulis* Engelm., occurs, but some data were secured within its range in the higher elevations of the coastal region in the vicinity of the Prohibition Claim and Tenquil plots.

The dates given for stage of development of the rust on each host are based upon observations actually made at the respective places or within the regions listed. When the earliest and latest dates were noted on, or within a day or so of, the date of actual development, such dates are included. When observations of earliest and latest development were not made on actual dates but could be determined approximately from previous or subsequent observations made at the respective points, the dates are given for the part of the month in which the stage of development occurred. The months are divided into 3 periods: *i.e.*, early, to mean from the 1st to the 10th of the month; mid, to mean from the 11th to the 20th, and late, to mean from the 21st to the last day of the month, inclusive, respectively.

One of the earliest dates for aecial development was in the spring of 1934. Besides the data for that year presented in table 1, it may be mentioned here that in the vicinity of Fernwood, Idaho, aeciospore dispersal was observed March 10, and that it was estimated the first aecia made their

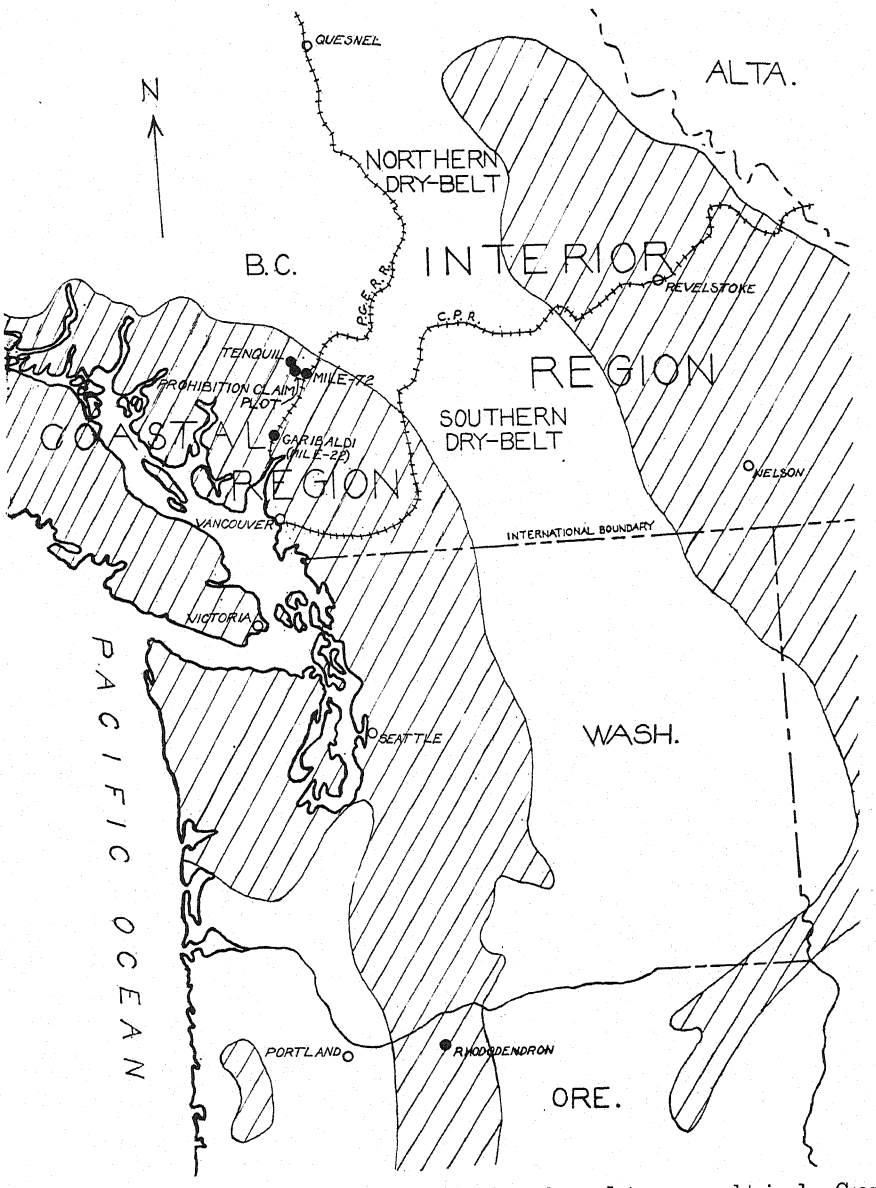


FIG. 1. Map showing regions and localities where data were obtained. Cross-hatched area outlines the botanical range of *Pinus monticola*. Solid black dots show localities referred to in tables 1 and 2.

appearance there in late February. Previous records for the State of Idaho have not been obtainable.

The data presented herein have been of value in analyzing the seasonal spread of the white pine blister rust in the Pacific Northwest. Lachmund[4] has shown the importance of time of aeciospore dispersal as a factor in influencing the degree and extent of seasonal spread of the rust.

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[4] Lachmund, H. G. Seasonal development of ribes in relation to spread of *Cronartium ribicola* in the Pacific Northwest. Jour. Agr. Res. 49: 93–114. 1934.

SEED TRANSMISSION OF HELMINTHOSPORIUM OF CORN[1]

W. D. VALLEAU

(Accepted for publication February 27, 1935)

In the publication "Corn Diseases in Illinois"[2] the authors discuss helminthosporium leaf blight of corn (*Zea mays*) caused by the fungus *Helminthosporium turcicum* Pass., but make no mention of the possibility that the pathogen is seed-borne. In the discussion of the "Seasonal Cycle of Parasite" (page 108) they state: "Nothing is definitely known concerning the form or manner in which the fungus remains alive during the winter. It is assumed, however, that it overwinters on the old crop refuse, and that in the following season the spores are carried by wind or other agencies to the new corn crop." That *Helminthosporium* sp. is commonly present in corn seeds seems not to have been discovered by these writers in their extensive studies of seed infection. Likewise, Manns and Adams[3] do not list Helminthosporium as one of the organisms isolated from 7,205 seeds cultured, but list it amongst fungi found (p. 508) "in several instances . . . internal of seed corn."

In studies conducted by the writer between 1920 and 1925 on corn-seed infection, evidence was obtained that *Helminthosporium* sp. was present in seed of essentially all ears examined. Failure of others to observe this fact is due to the methods commonly used in studies of seed infection. In 1926 Valleau and Johnson[4] stated "In numerous tests conducted by the senior writer during the past five years, it has been determined that whereas the seeds of certain ears might appear to be free from fungi in germinator tests and culture dish tests conducted for a period of 6 or 8 days, yet when grown in sterile sand for longer periods, the presence of *F. moniliforme* and some others of the common seed-borne organisms could usually be demonstrated. *Helminthosporium* sp. is rarely found in germination tests of corn seeds or in cultures made from crushed seed, but if seedlings are grown in sand in an ordinary room and allowed to remain until they die, *Helminthosporium* sp. is quite commonly found fruiting on the stem and seed coat remnants." The object of the present note is to record the evidence for seed infection with *Helminthosporium* sp.

[1] The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the Director.

[2] Koehler, Benjamin, and James R. Holbert. Corn diseases in Illinois. Ill. Agr. Expt. Sta. Bul. 354. 1930.

[3] Manns, Thomas F., and J. F. Adams. Parasitic fungi internal of seed corn. Jour. Agr. Res. 23: 495–524. 1923.

[4] Valleau, W. D., and E. M. Johnson. A method of growing corn seedlings (*Zea mays*) free from seed-borne organisms. Jour. Agr. Res. 32: 1195–1198. 1926.

If corn seeds are planted in clean sand in a room with reduced light and kept at a temperature of about 80° F., germination is rapid and the second node or that from which the first permanent roots are derived is carried above the surface of the sand often as much as 3 inches. As a consequence, death of the seminal roots due to seed-borne organisms results in death of the plant; while in the field, by the time the seminal roots are destroyed, the permanent roots are well established. Death of seedlings occurs early or late depending perhaps upon the available food supply and upon the readiness with which seed-borne organisms are able to grow out from between the pericarp layers. This, in turn, appears to depend, to quite an extent at least, on the texture of the seed and the compactness of the pericarp layers. Seed-borne organisms develop readily from seeds that are starchy and have relatively loose pericarp layers. Seeds that are flinty, with closely compacted pericarp layers produce relatively long-lived seedlings in the sand box, and the fungi require a relatively longer time before they escape from the pericarp and attack the seminal roots and mesocotyl. Left a sufficiently long time under these conditions, nearly every corn seedling will die from the attack of seed-borne organisms. The longevity of sand-box seedlings from a given ear in comparison with that of seedlings from other ears, appears to be an inherited character and not an indication of the degree to which the seeds carry pathogenic organisms, as shown in table 1 where the seedlings of 3 "long-lived seedling" strains are compared with 3 "short-lived seedling" strains for 3 successive years, in self-pollinated lines of Boone County White.

TABLE 1.—*Length of life in the sand box, of 6 strains of Boone County White corn self-pollinated for 3 successive years.*

	Long-lived			Short-lived		
Strains No.	1	2	3	4	5	6
Selfed once	9.5	8.4	8.4	2.6	2.6	2.6
Selfed twice	9.0	9.7	11.1	1.4	2.1	1.9
Selfed three times	15.4	18.1	15.5	10.2	9.3	5.7

The longevity figure is the average number of days that seedlings lived in the sand box after death of the first plant. It will be noted that the short-lived strains were consistently short-lived through the 3 successive self-pollinated generations and the long-lived strains were consistently long-lived through the 3 self-pollinated generations. There was no evidence that length of life was associated with degree of infestation of the seeds with fungi.

Seeds from which the organisms grow readily, develop extensive dis-

colorations of the pericarp relatively early, while seeds from which the fungi grow slowly develop discolorations later and less extensively. The discolorations may be nearly completely prevented by treating the seeds with Bordeaux mixture, and may be aggravated by soaking the seed in 1 part of CaO to 4 parts of water for 20 hours. This treatment has a tendency to break down the seed coats and release the organisms. Seeds free from *Gibberella saubinetii* and *Diplodia zeae* develop various shades of red, bright green, dark olive, coal black, and a dirty brown. The various shades of red usually indicate the presence of *Fusarium moniliforme*. The cause of the bright green color has not been determined. Dark olive is commonly produced by *Alternaria* sp. and the black areas appear to be caused by *Helminthosporium* sp. A fungus that produces minute brownish sclerotia and develops a brown growth on potato agar was isolated from the dirty brown seed coats.

The discolorations appear to develop in areas of the seed coats already penetrated by the fungi before the seed is planted. Manns and Adams claimed that seed infection was largely limited to the area about the cap, but the writer has demonstrated by a study of serial paraffin sections that the fungi are distributed in the seed coats from one end of the seed to the other and are not at all limited to the portion near the point of attachment of the seed to the cob.

Seed infection evidently takes place early in the development of the embryo, as, in the extensive study of the time of infection of corn seeds, the writer found that ears harvested in late milk stage were as heavily infected, as indicated by seed coat discoloration and death of seedlings in the sand box, as seeds from matured and properly cured ears. From these studies and from studies of serial sections of corn seeds, it appears probable that infection occurs through the silks soon after pollination and that the fungi penetrate between the pericarp layers, in some cases developing sclerotium-like masses, before the pericarp walls have completed their development. It was not until many hundreds of seedlings had been grown in the sand box that it was realized that *Helminthosporium* sp. was a rather common seed-borne organism and that its presence was indicated by coal-black markings on the seed coats.

In a study of 500 ears that had been selected in the field and cured with heat, the smoothest and the roughest 50 ears were selected. From the smooth ears, 15 that produced the longest-lived plants in the sand box were selected and from the rough ears 15 that produced the shortest-lived plants. These were to be tested for yield in the field.[5] A study was then made of

[5] When tested in duplicate in the field, the increase in yield of smooth ears over rough ears was 39.43 per cent. This was attributed to physical characters of the plants and not to differences in seed infection. The pericarp layers are considered botanically

the organisms contained in the seeds of the 15 smooth ears, which would ordinarily have been considered the most nearly fungus-free. Twenty seeds from each ear were treated 3 minutes in 95 per cent alcohol; then 15 minutes in 1:1000 mercuric chloride solution, after which they were immediately planted in 2 series in sand that had been sterilized 35 minutes under 22 pounds' pressure. The seeds were set 1 inch apart in rows 2 inches apart. After planting, the sand was watered with sterile water and not watered again until near the end of the test when only a few plants remained. One series of plants was removed and examined between the 20th and 23rd days. If fruiting bodies were present on the seed coats these were recorded; if not, the seed coats were washed in sterile water and placed in a culture dish to be examined for fruiting bodies at the end of 24 or 48 hours. The other series was allowed to remain in the sand until the plants were dead, when the plants were removed and the fungi fruiting on the seed coats or mesocotyl determined by direct observation or by culturing.

Of 269 plants or seed remnants examined 94, or 35 per cent, had *Fusarium moniliforme* fruiting on them; 32 from 13 of the 15 ears, or 11.9 per cent, had *Helminthosporium* sp. fruiting abundantly either on the black areas on the seed coats or on the mesocotyl, often blackening it completely with a palisade of spores. On 11, Alternaria was fruiting; on 80 others, miscellaneous fungi were found. In the examinations it was found that *Helminothosporium* sp. was constantly associated with the coal-black areas and it, therefore, seems probable that black markings on the pericarp of seeds in the sand box are an indication of the presence of Helminthosporium.

In looking over earlier records on pericarp discoloration black markings were recorded on seed from 88 of 109 ears of Boone County White and from 73 of 76 ears of Reid's Yellow Dent. A total of 28.5 per cent of 2058 seeds of Boone County White and 47.4 per cent of 1069 seeds of Reid's Yellow Dent developed black markings, suggesting the presence of Helminthosporium.

It appears from these studies and from test-tube and culture-plate studies, that organisms between the pericarp layers are often sealed and not capable of growing until the pericarp walls are broken down by other processes than those occurring during germination under aseptic conditions. It seems evident that the usual culture-plate method cannot be relied upon for the accurate determination of organisms carried by seed.

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to be a leaf folded about the ovary. Therefore, long, deep-dented seeds should be borne on large-leaf plants inefficient in the use of water, and small, flinty seeds on plants with relatively smaller leaves, more efficient in the use of water.

PHYTOPATHOLOGICAL NOTES

Some Factors Affecting Infection With and Spread of Diplodia zeae in the Host Tissue.—A better knowledge of factors affecting the infection and subsequent development of *Diplodia zeae* (Schw.) Lév. in the tissues of corn, *Zea mays* L., is important in connection with any study dealing with the cause of resistance to this fungus.

The following observations, though detached and fragmentary, may prove suggestive to those interested in the study of this fungus and its host relationships. They are presented as a possible lead for future investigations of a disease that has yielded relatively few tangible leads in the past.

In general, stalk tissues become more predisposed to *Diplodia zeae* as the grain approaches maturity. Conditions that predispose stalk tissues to *D. zeae* and those that predispose the ear and grain to the fungus are not always the same. Moreover, resistance of one set of tissues of the corn plant to *D. zeae* does not insure resistance of other parts of the same plant.

In 1931 stalk tissues of certain inbred lines not permitted to set grain were invaded less rapidly and to a less extent following hypodermic injection of a spore suspension of *Diplodia zeae* than were stalk tissues of comparable plants of the same inbreds allowed to fruit normally. The inoculations were made in the pith tissues of the third or fourth internodes above the soil line 3 weeks after the pollination period.

The stalk tissues of plants whose leaves were injured by bacterial leaf blight the latter half of August, during the epidemic of that disease in field corn in 1932, were found to be invaded by Diplodia, prior to maturity of the grain, to a significantly greater extent than were the stalk tissues of comparable plants only small areas of whose leaves had been killed by bacterial leaf blight.

Artificial defoliation experiments have been conducted from 1932 to 1934, inclusive. During the last 10 days in August, from 25 to 35 per cent of the tip end of each leaf of part of the plants was removed by clipping. Stalk tissues of these partly defoliated plants were invaded earlier and to a much greater extent by *Diplodia zeae,* following both natural infection and hypodermic injection of a spore suspension while the leaves were still green, than were stalk tissues of comparable plants none of whose leaves were clipped. A summary of the results for the 3-year period is given in figure 1.

Infestations of second-brood chinch bugs, which feed very largely on the carbohydrates they remove from the lower parts of the corn plant, have been found to be followed by a marked increase in the rate at which *Diplodia zeae* invades the stalk tissues prior to maturity, excepting in strains highly resistant to injury from the attack of second-brood chinch bugs. During 1933

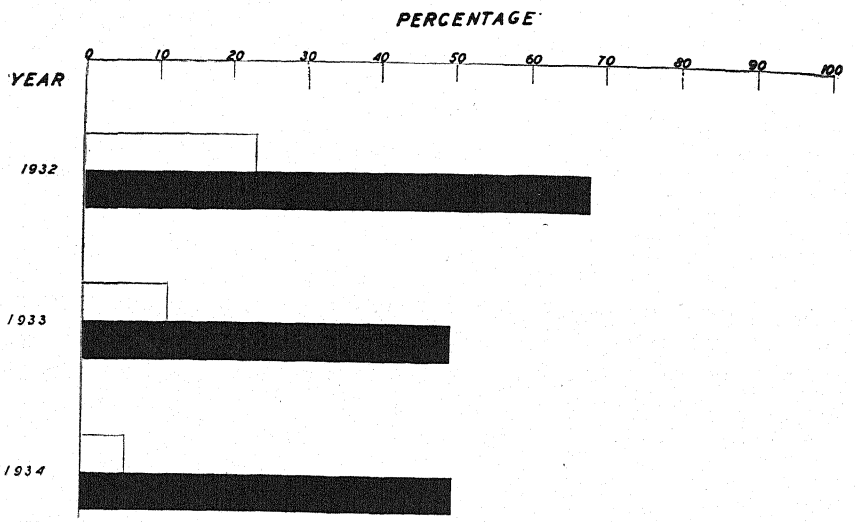


FIG. 1. Percentage of plants at maturity with stalks conspicuously rotted by Diplodia in plant populations whose leaves were not clipped (white bar) and in plant populations whose leaves were partly clipped (black bar), Bloomington, Illinois, 1932–1934, inclusive.

and 1934 the amount of grain damaged by *D. zeae* was almost always very much greater where the corn plants were infested by second-brood chinch bugs, but not killed prior to maturity, than where comparable plants were not infested with second-brood chinch bugs.

Johnson and Christensen[1] have reported that ears borne on smut-infected plants were more likely to be damaged by ear rots than ears borne on smut-free plants.

Stalk and shank tissues, and also the ears, of plants exposed to both naturally occurring and artificially produced chilling and freezing temperatures, as they approach complete maturation, usually have been invaded more rapidly and to a much greater extent by *Diplodia zeae* than have the same tissues of comparable plants not exposed to such temperatures.

In each case cited, increased susceptibility of the stalks to the fungus is associated with conditions that may well result in a reduction of the carbohydrate reserves of the plants. Apparently this situation does not hold, however, for susceptibility in the ears.—J. R. HOLBERT, P. E. HOPPE, and A. L. SMITH (Cooperative investigations between the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Illinois and Wisconsin agricultural experiment stations).

[1] Johnson, I. J., and J. J. Christensen. Relation between number, size and location of smut infections to reduction in yield of corn. Phytopathology 25: 223–233. 1935.

A Top-necrosis Virus Found in Some Apparently "Healthy" Potatoes.[1]—Murphy and McKay[2] found in their study on a comparison of some European and American diseases of the potato that they had considerable difficulty in introducing certain American viroses into healthy President plants. There was found to be throughout the American material a latent virus or viruses, which had a severe necrotic effect on President. They found that this latent virus (or more probably viruses) was present in the "healthy" Green Mountain, as well as in the obviously diseased plants.

The writer has made grafts of mild mosaic, crinkle mosaic, and leaf-rolling mosaic from Earliest of All and Bliss Triumph on healthy Arran Victory and President. In many cases top necrosis developed on these two varieties. A small number of grafts of scions from "healthy" Burbank and Earliest of All were made to President, and some of these plants developed a top necrosis. These symptoms could not be ascribed to the so-called latent mosaic of X-virus, which is universally present in healthy American commercial potatoes, since that virus produces in these two varieties only a mosaic type of mottling without necrosis.

In 1933 the writer received several diseased and healthy tubers of different European varieties from R. N. Salaman of England. Among the tubers was the variety Up-to-Date, which is a symptomless carrier of virus B. Bawden, according to Smith and Brooks,[3] states that the streak-producing B-virus is almost universal in this variety, and is usually accompanied by virus X, (the latent mosaic of "healthy" commercial potatoes). Grafts from Up-to-Date to Arran Victory and President produced a top necrosis.

In order to determine whether or not the X-virus is required to produce this disease, grafts of Up-to-Date were made to the U. S. D. A. Seedling No. 41956, which is resistant to X-virus.[4] By this procedure it is possible to remove the X-virus and to retain the B-component. Grafts of the infected seedling to Arran Victory produced typical top necrosis symptoms. Grafts of the infected seedlings to tomato transferred the B-virus, but failed to develop any noticeable symptoms. The presence of the B-virus was demonstrated when grafts of the infected tomato plants produced top necrosis in Arran Victory and President.

[1] This paper is based upon investigations carried on as a cooperative project between the Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Oregon Experiment Station at Corvallis, Oregon.

[2] Murphy, Paul A., and R. McKay. A comparison of some European and American virus diseases of the potato. Sci. Proc. Roy. Dubl. Soc. 20: 347–358. 1932.

[3] Smith, K. M. (Foreword by F. T. Brooks). Recent advances in the study of plant viruses, 423 pp. P. Blakiston's Sons. Philadelphia, 1934. (See page 316).

[4] Schultz, E. S., and W. P. Raleigh. Resistance of potato to latent mosaic (Abstr.) Phytopathology 23: 32. 1933.

Several grafts of Up-to-Date were made to the following American varieties: Burbank, White Rose, Irish Cobbler, Earliest of All, and Bliss Triumph. Symptoms failed to develop either during the current season or in the progeny. These results indicate that the American varieties tested probably serve as symptomless carriers of virus B, although return grafts from grafted American varieties to President or Arran Victory have not yet been made, because of insufficient plants of these varieties.

Since the American varieties tested fail to develop symptoms when grafted with virus B, and since this virus produces top necrosis in Arran Victory and President, it is believed that the virus component found in apparently healthy tubers of some American varieties in addition to virus X, is the same as Bawden's virus B.

Preliminary results indicate that not all varieties of American potatoes carry this virus, since in some cases a mottling without top necrosis resulted when grafts of scions of apparently healthy American potatoes were made on Arran Victory or President. Plans are being made to test a large number of plants of American varieties for the presence of this top-necrosis virus. Although European workers have referred to the presence of virus B in American varieties, this fact has not previously been confirmed by American investigators. This is undoubtedly due to the fact that most, if not all, or our American varieties are symptomless carriers of this virus and, since it fails to produce any apparent symptoms on tobacco or Datura, it is difficult to detect. When it is introduced into European varieties, like President, Arran Victory, or Epicure, the presence of this virus can be readily demonstrated. Further studies of this virus component are in progress.—T. P. DYKSTRA, *Oregon State College,* Corvallis, Oregon.

An Apple Canker Caused by Monochaetia mali.—An apple canker caused by *Monochaetia mali* (E. and E.) Sacc. (fig. 1) has been occasionally seen by the writer since 1910. In May, 1911, successful inoculations were made using spores from pure cultures, and in October of the same year the fungus was reisolated from the resulting typical cankers. *Monochaetia mali* fruited copiously on cankers produced from artificial inoculations, as it does on those occurring naturally. The fungus enters through deep wounds, grows deeply into the wood, and subsequently attacks the resulting wound callus, producing numerous fruiting bodies on both the exposed wood and the layer of wound callus. The killing of successive layers of wound callus (Fig. 1) results in the formation of a type of canker somewhat resembling the European apple canker caused by *Nectria galligena* Bres. Since the canker caused by *Monochaetia mali* appears to be uncommon, it cannot be considered as a serious apple disease.—JOHN W. ROBERTS, Division of Fruit and Vege-

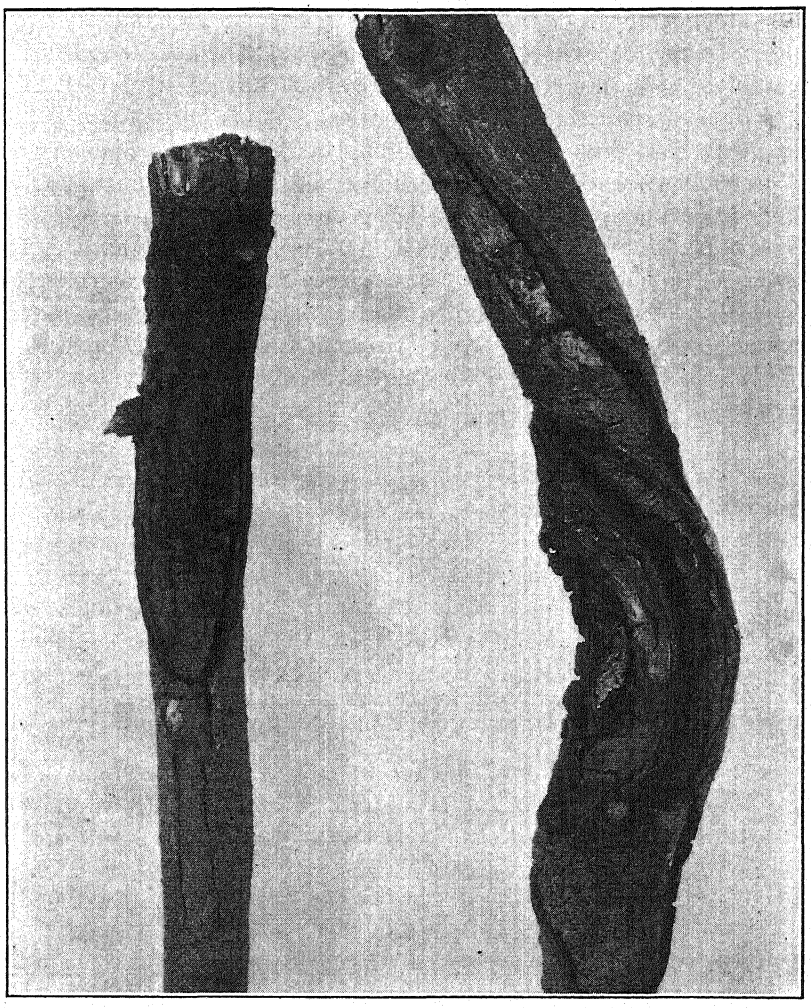


FIG. 1. An apple branch in two pieces, showing a canker caused by *Monochaetia mali*. The canker on the smaller piece is a continuation of that on the larger piece. Note killing of successive callus layers, spread of canker, and presence of fruiting bodies. Collected by Leslie Pierce, Vincennes, Ind., June, 1934. ×1.

table Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

Notes on the Occurrence of Tuberculina maxima on the Aecia of Cronartium cerebrum.—In a recent article Hubert[1] listed *Cronartium cerebrum*

[1] Hubert, E. E. Observations on *Tuberculina maxima*, a parasite of *Cronartium ribicola*. Phytopath. 25: 253–261. 1935.

(Peck) Hedgc. and Long as one of the hosts of *Tuberculina maxima* Rostrup. In the collections of the Division of Forest Pathology, Bureau of Plant Industry, Washington, D. C., there are 225 naturally infected specimens of *C. cerebrum* occurring on 14 species of Pinus in the eastern United States. Only 7 of these specimens are infected with *T. maxima*. All 7 are on *P. virginiana* from Maryland, Virginia, and Pennsylvania. The above-mentioned specimens of the rust, a large number of which were collected by the writer, are typical of thousands of *C. cerebrum* galls that the writer has observed during the past 30 years. Since *T. maxima* was collected so infrequently, it must be concluded that it is rare and of no economic importance on *C. cerebrum*.—GEO. G. HEDGCOCK, *Division of Forest Pathology, Bureau of Plant Industry,* Washington, D. C.